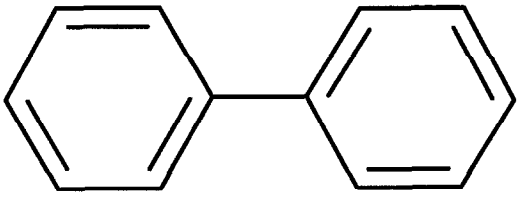
201-14973B

Biphenyl

CAS Number 92-52-4



Data Set

Existing Chemical	:	ID: 92-52-4
CAS No.	:	92-52-4
EINECS Name	:	biphenyl
EC No.	:	202-163-5
TSCA Name	:	1,1'-Biphenyl
Molecular Formula	:	C12H10

Producer related part

Company	:	**SOCMA Biphenyl Working Group**
Creation date	:	30.10.2003

Substance related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	30.10.2003
Status	:	
Memo	:	
Printing date	:	18.12.2003
Revision date	:	
Date of last update	:	18.12.2003
Number of pages	:	54
Chapter (profile)	:	
Reliability (profile)	:	
Flags (profile)	:	

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	SOCMA Biphenyl Working Group
Contact person	:	John Murray
Date	:	
Street	:	SOCMA
Town	:	1850 M Street NW, Suite 700, Washington, DC 20036
Country	:	
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

30.10.2003

1.2 SYNONYMS AND TRADENAMES

2.1 MELTING POINT

Value : = 69 - 71 °C

Test substance :
Biphenyl, CASNO 92-52-4
Reliability : (2) valid with restrictions

Handbook data are assigned reliability of 2
Flag : Critical study for SIDS endpoint
30.10.2003 (22)

2.2 BOILING POINT

Value : = 254 - 255 °C at 1010 hPa

Test substance :
Biphenyl, CASNO 92-52-4
Reliability : (2) valid with restrictions

Handbook data are assigned reliability of 2
Flag : Critical study for SIDS endpoint
30.10.2003 (22)

2.4 VAPOUR PRESSURE

Value : = .0119 hPa at 25 °C

Test substance :
Biphenyl, CASNO 92-52-4
Reliability : (2) valid with restrictions

Published data are assigned reliability of 2
Flag : Critical study for SIDS endpoint
30.10.2003 (8)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : = 4.01 at 25 °C
pH value :

Test substance :
Biphenyl, CASNO 92-52-4
Reliability : (2) valid with restrictions

Published data are assigned reliability of 2
Flag : Critical study for SIDS endpoint
30.10.2003 (17)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 7.28 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	

Remark :

This result is supported by a second experimental value found in the EPIWIN 3.05 database as:

```
Experimental Water Solubility Database Match:
  Name     :  BIPHENYL
  CAS Num  :  000092-52-4
  Exp WSol :  6.94 mg/L (25 deg C)
  Exp Ref  :  PEARLMAN,RS ET AL. (1984)
```

This value is also supported by a measured value of 7.3 mg/L at 24.6 C reported by RD Wauchope and FW Getzen, Temperature Dependence of Solubilities and Heats of Fusion of Solid Aromatic Compounds. J Chem Eng Data 17:38 (1977)

Test substance :

Biphenyl, CASNO 92-52-4

Reliability : (2) valid with restrictions

Handbook data are assigned reliability of 2

Flag : Critical study for SIDS endpoint

06.11.2003 (26)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	= .0000000000072 cm³/(molecule*sec)
Degradation	:	ca. 50 % after 18 hour(s)

Method :

INDIRECT PHOTOLYSIS:

Initial estimate based on AOP program in EPIWIN. The results of this calculation are shown below. There was also a match in the experimental reaction rate data base and this esperimental rate constant is shown below. There is a good correlation between the estimated reaction-rate constant and the experimental value.

AOP Program (v1.90) Results:
==========
SMILES : c1ccccc1c2ccccc2
CHEM : Biphenyl
MOL FOR: C12 H10
MOL WT : 154.21

```
------ SUMMARY (AOP v1.90): HYDROXYL RADICALS --------------
Hydrogen Abstraction       =   0.0000 E-12 cm3/molecule-sec
Reaction with N, S and -OH =   0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds   =   0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds =   0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings =   6.7747 E-12 cm3/molecule-sec
Addition to Fused Rings    =   0.0000 E-12 cm3/molecule-sec

   OVERALL OH Rate Constant =   6.7747 E-12 cm3/molecule-sec
   HALF-LIFE =     1.579 Days (12-hr day; 1.5E6 OH/cm3)
   HALF-LIFE =    18.946 Hrs

-----SUMMARY (AOP v1.90): OZONE REACTION --------------------
        ******  NO OZONE REACTION ESTIMATION ******
       (ONLY Olefins and Acetylenes are Estimated)

Experimental Database Structure Match:
  Chem Name :  Biphenyl
  CAS Number:  000092-52-4
  Exper OH rate constant   :  7.2  E-12  cm3/molecule-sec
    Exper OH Reference:  ATKINSON,R (1989)
  Exper Ozone rate constant:  < 2.0 E-19  cm3/molecule-sec
  Exper NO3 rate constant  :  ---  cm3/molecule-sec
```

DIRECT PHOTOLYSIS: Biphenyl shows very little absorption of light at wavelengths greater than 290 nm, therefore, direct photolysis of the compound in air is unlikely to be an important process*

* (Moore WM et al; Soil Phase Photodegradation of Toxic Organics at Contaminated Disposal Sites for Soil Renovation and Groundwater Quality Protection. USGS Report No. G-1304, Reston, VA. NTIS PB-89-237267, Springfield, VA (1989) as cited in National Library of Medicine Hazardous Substance Data Base, Last Revision Date: 20020806)

Result :

Direct: No photolysis expected

Indirect: Estimated half-life is ca 18 hours based on the calculated or the experimentally determined hydroxyl radical rate constant with Biphenyl

Test substance :

Biphenyl, CASNO 92-52-4

Reliability : (2) valid with restrictions

Estimate based on reliable reaction rate constant.

Flag : Critical study for SIDS endpoint

13.12.2003 (10)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : > 1 year at 25 °C
t1/2 pH9 : at °C
Deg. product :
Method : other: estimated on chemical principles
Year :
GLP :
Test substance :

Method :

Estimate using chemical principles

Result :

Molecule does not contain a water-reactive or hydrolysable group. The following are considered water stable for this reason:

-Benzenes
-Biphenyls
-.....

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Stable in water indefinitely

Reliability : (2) valid with restrictions

Estimate based on valid chemical principles and from EPIWIN are assigned a reliability of 2

Flag : Critical study for SIDS endpoint

30.10.2003 (19)

3.3.2 DISTRIBUTION

Media : air - sediment(s) - soil - water
Method : Calculation according Mackay, Level III
Year :

Method :

Measured values for physical values of Biphenyl were input into EPIWIN as shown below. Default biodegradation rates were determined to be in reasonable accord with experimental values. Model was allowed to assume equal distributions to air, water and soil. EQC Level model (as found in EPIWIN 3.05) was utilized.

Result : Results of the Level III fugacity modeling are:

Level III Fugacity Model (Full-Output):

```
=====================================
 Chem Name    : Biphenyl
  Molecular Wt: 154.21
  Henry's LC  : 0.000308 atm-m3/mole (Henry database)
  Vapor Press : 0.0089 mm Hg  (user-entered)
  Liquid VP   : 0.0248 mm Hg  (super-cooled)
  Melting Pt  : 70 deg C (user-entered)
  Log Kow     : 4.01  (user-entered)
  Soil Koc    : 4.2e+003  (calc by model)

       Concentration   Half-Life    Emissions
         (percent)        (hr)       (kg/hr)
Air       5.54            35.7         1000
Water     28.8            360          1000
Soil      63.8            360          1000
Sediment  1.91            1.44e+003    0

         Fugacity    Reaction  Advection  Reaction    Advection
           (atm)      (kg/hr)    (kg/hr)   (percent)   (percent)
Air       7.09e-011    870       448        29          14.9
Water     2.31e-009    448       233        14.9        7.76
Soil      5.67e-010    993       0          33.1        0
Sediment  7.59e-010    7.44      0.309      0.248       0.0103

   Persistence Time: 270 hr
   Reaction Time:    349 hr
   Advection Time:   1.19e+003 hr
   Percent Reacted:  77.3
   Percent Advected: 22.7

   Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin):
      Air:      35.66
      Water:    360
      Soil:     360
      Sediment: 1440
        Biowin estimate: 2.902  (weeks)

   Advection Times (hr):
      Air:      100
      Water:    1000
      Sediment: 5e+004
```

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Under conditions of equal initial distribution to water, soil and air, Biphenyl is expected to distribute preferentially in soil > water > air > sediment.

Reliability : (2) valid with restrictions

Estimate based on valid chemical principles and from EPIWIN are assigned a reliability of 2

Flag : Critical study for SIDS endpoint

30.10.2003 (9)

3.5 BIODEGRADATION

Type : aerobic
Inoculum : other: Natural river water
Concentration : 1 µg/l related to Test substance
100 µg/l related to Test substance
Contact time : 16 day(s)
Degradation : > 80 - 95 (±) % after 8 day(s)
Result :

Method :

Test water was collected from the Titabawassee river in Michigan upstream of any significant industrial or municipal discharge, filtered through course filter paper and used for testing within four hours of collection. Test material was dissolved directly in river water without use of a carrier by evaporating a hexane solution of Biphenyl on the inside surface of a glass jar and adding river water to the jar and rolling the jar to dissolve the test material. Serial dilutions of this stock were made to achieve the lower concentrations.

Two methods were used to estimate biodegradation. Hplc analysis of methylene chloride extracts of the incubation mixtures and evolution of carbon dioxide. Cabon-14 radiolabeled Biphenyl was utilized for the carbon dioxide evolution studies and carbon dioxide was trapped in ethanolamine and 2-methoxyethanol and determined by liquid scintillation counting.

The bacteria population of the river water was estimated using Millipore Total Count paddles incubated for three days before counting. An average of 6900 CFU/mL was determined from water collected on March 10, 1980 and used for some of the biodegradation studies.

Result :

Concentrations of 1, 10 or 100 micrograms (ug)/L of Biphenyl were tested with freshly-collected river water on one day. Carbon dioxide evolution was rapid with estimated 50% evolution of carbon dioxide occurring after 1.5, 2 and 3 days of incubation in the dark at 20 C. This was confirmed using water collected on another day, which gave a 2.5-day 50% evolution of total carbon at the 1-ug/L concentration of Biphenyl.

Carbon dioxide evolution was measured from the Biphenyl degradation studies on days 1, 2, 3, 4, 8, and 16. Results of Biphenyl degradation are presented graphically in the publication and show 60% or greater carbon dioxide evolution at 4-day sampling time for concentrations of 1, 10 and 100 ug/L. The best recovery was obtained at 100 ug/L (where the bacteria take up a smaller percentage of the organic carbon) where 80% evolution was obtained at 8 days and ca 97% at 16 days. Carbon dioxide evolution curves were similar at the lower concentrations of test substance but recovery of carbon dioxide was only about 80 % at 1 and 10 ug/L. At all

concentrations, the biodegradation appeared to be complete by 16 days after start.

Parent compound was also monitored by HPLC after methylene chloride extraction. Loss of Biphenyl was rapid, showed no induction period, and was essentially complete (less than ca 5% remaining) after 4 days of incubation. Identification of a metabolite at about the 2 to 5% level of parent was made but the metabolite was not identified.

Material balance studies were conducted to determine the total recover of radiolabeled carbon by measuring the amount of radioactivity remaining in the river water after carbon dioxide evolution and extraction of parent material and metabolites with methylene chloride. Individual material balances are not given but it was determined that the mean accountability of carbon-14 for all studies conducted in this publication (including studies with chlorinated biphenyls) was 92.2%. It was determined that the typical loss of 5% of the radiocarbon came from purging of equipment during carbon dioxide measurements and volatilization during setup.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Biphenyl is rapidly biodegraded to carbon dioxide in typical river water from an area that drains primarily agricultural activities and is upstream from major industrial or municipal effluents. The half-life in river water is on the order of 2 days.

Reliability : (1) valid without restriction

A carefully conducted and well-documented publication from a GLP study.

Flag : Critical study for SIDS endpoint

13.12.2003 (6)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	192 hour(s)
Unit	:	mg/l
NOEC	:	= .17 measured/nominal
LC50	:	= 1.3 measured/nominal
Limit test	:	
Analytical monitoring	:	yes
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

Animals: Rainbow trout (Salmo gairdneri Richardson) used in acute testing were obtained as eyed embryos from Mt. Lassen Trout Farms, Red Bluff California on March 11, 1987. Upon arrival they were placed in a trout hatcher and incubated at 12 ± 2°C until hatched. Juveniles were held in 110 L stainless steel aquaria at a water temperature of 12 ± 2°C, and were provided a 16-h light/8-h dark photocycle. A synthetic diet was provided ad libitum. Juvenile trout ca. 160 days post-hatch were acclimated to test temperature at least 72-h prior to testing.

The 192-hour flow-through acute test was conducted with juvenile rainbow trout. There were six test concentrations, an acetone control with the acetone concentration equaling the highest concentration in any treatment group (0.1 ml/L) and a water control. Each test concentration and control was set in duplicate with each replicate containing 10 fish. During each cycle of the diluter, 1 L of test solution or water was delivered to each replicate.

Water: The water supply is pumped from the Upper Saginaw Bay of Lake Huron. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. As it enters the laboratory, the water is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the test; pH 7.5 to 7.8 hardness (mg/L as calcium carbonate) 73 to 76 alkalinity (mg/L as calcium carbonate) 48 to 54 and conductivity 150 to 160 (umhos/cm).

Dilutor: An intermittent-flow proportional diluter system was used. This system was designed to deliver six test concentrations, a carrier and water control. The diluter was calibrated so that the concentration of the test material in each treatment below the high concentration was approximately 65 percent of that in the next higher treatment level. The carrier control received acetone at a concentration equaling the highest concentration in any treatment group, (no more than 0.1 ml/L). The diluter operates as follows: a precision dosing system delivers the test material from a stock bottle to a mixing chamber where it is mixed with dilution water and then distributed to "toxicant cells". When the diluter cycles, the test material from each toxicant cell blends with water from its respective dilution water cell and then flows into mixing/splitting chambers. Silicone delivery tubes from these chambers provide approximately 500 mL to the test aquaria, which are positioned on one tier, side by side, in a temperature-controlled water

trough.
The diluter was calibrated prior to the beginning of the tests and was found to be operating normally. The diluter was set to provide at least 15 volumes turnovers in the test aquaria each twenty-four hours.
The test vessels were constructed of double-strength glass glued with clear silicone adhesive, and measure approximately 30 x 15 x 14 cm deep. Each is provided with a nylon screen covered drain that maintains a water volume of 3.7 liters. In the embryo-larval test, the embryos were incubated in circular (124 mm in diameter by 51 mm high) cups with 360 um nylon screen bottoms that were supported in the test vessels by glass beads. The flow from the delivery tube was directed into the incubation cup to produce a flow of water around the embryos during the incubation period.

Statistics: The flow-through acute concentration-mortality data were analyzed for daily LC50 values. A computer program was used to calculate the LC50 values and corresponding 95% confidence intervals. (Stephan, U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota). This program has three methods available; probit analysis, moving average angle analysis, and binomial probability.

Result :

Analyzed biphenyl concentration ranged between 83 and 110 percent of nominal. The 192-hour LC50 based on average measured concentrations, was determined to be 1.3 mg/L. The 24 through 120-h LC50 values were not determined due to insufficient mortality. Sublethal effects such as complete loss of equilibrium (immobile on bottom) or partial loss of equilibrium (the inability to maintain normal swimming posture) were observed at 0.81 mg/L and higher. Anorexia was observed at 0.60 mg/L and higher; and, melanosis and long fecal casts were observed at 0.27 mg/L and higher.

		PERCENT DEAD AT (hours)							
Conc (mg/L)	No Fish	24 --	48 --	72 --	96 --	120 ---	144 ---	168 ---	192 ---
1.502*	20	10	10	15	40	45	60	65	70
0.812*	20	0	0	0	0	0	0	0	0
0.604*	20	0	0	0	0	0	0	0	0
0.373*	20	0	0	0	0	0	0	0	0
0.272*	20	0	0	0	0	0	0	0	0
0.171	20	0	0	0	0	0	0	0	0
0.000	20	0	0	0	0	0	0	0	0

* = Sublethal effects

Analytically determined concentrations

Day 0 (mg/L)	Day 3 (mg/L)	Day 8 (mg/L)	Mean ± S. D. (n = 4 or 5)
1.539	1.305	1.381	1.502 ±0.15
0.886	0.830	0.743	0.812 ±0.061
0.595	0.592	0.623	0.604 ±0.029
0.403	0.361	0.380	0.373 ±0.024
0.282	0.259	0.280	0.272 ±0.011
0.171	0.154	0.174	0.171 ±0.015

Test condition : ----------CONDITIONS----------

Temperature	12 ± 1°C
Photoperiod	16 hrs light/8 dark
Aeration	None
Type of Test	Acute
Diet	None 1st 96 hours; once daily thereafter
Test Vessel Size	Approximately 30x15x14 cm deep
Test Volume	3.7 L
No. of Treatment groups	6
No. of Replicates/Treatment	2
Organisms/Replicate	10
Observations	D.O., pH, temperature morality, sublethal effects
Effect Criteria	Sublethal effects and mortality
Length of Test	192 hours
Mean wt of fish	0.647 g
Dissolved Oxygen	>83% Saturation (7.7 - 9.0 mg/L)

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The 192-hour LC50 for biphenyl under these conditions is 1.36 (0.81-1.5) mg/L
The 192-hour NOEC for biphenyl under these conditions is 0.17 mg/L

Sublethal effects were noted at 0.27 mg/L and higher during the test

Reliability : (1) valid without restriction

High quality guideline-like study under GLP with analytical support

Flag : Critical study for SIDS endpoint

14.12.2003 (7)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : flow through
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC0 : = .04 measured/nominal
EC50 : = .36 measured/nominal
EC100 : > .96 measured/nominal
Limit Test : no
Analytical monitoring : yes
Method :
Year :
GLP : yes

Method :

Animals: Daphnia magna Straus, 1820, was used as the test organism in this study. The daphnids were cultured in the laboratory from parthenogenetic females. On the day before testing began, reproductively mature females were isolated. Young produced by these adults were collected and used for testing within 24 hrs.

The acute flow-through toxicity test consisted of exposing groups of 10 neonate daphnids to five concentrations of the test material, a carrier control (acetone 0.1 ml/L) and a water control. The five test concentrations and the controls were set in triplicate, resulting in 30 neonate daphnids being exposed to each concentration. The test vessels were maintained in a temperature

controlled water trough set at 20±1°C. Dissolved oxygen, pH and temperature were measured in the high, middle, low and control concentrations daily. The duration of this test was 48 hrs.

Water: The water supply is pumped from the Upper Saginaw Bay of Lake Huron. The water is limed and flocculated with ferric chloride by the City of Midland water treatment plant. As it enters he laboratory, the water is sand filtered, pH adjusted, carbon filtered, and U.V. irradiated prior to use. The water had the following range of analyses during the test; pH 7.4 to 7.7, hardness (mg/L as calcium carbonate) 73 to 78, alkalinity (mg/L as calcium carbonate) 49 to 52 and conductivity (umhos/cm) 160 to 170.

Dilutor: Testing was conducted with an intermittent-flow proportional diluter equipped with a Micromedic automatic pipette, which was triggered to inject the appropriate amount of test material into the toxicant mixing chamber at the beginning of each cycle. The toxicant mixing chamber was equipped with a recirculating pump that provided mixing for at least three minutes before the solution was delivered to the testing chambers. The diluter had a dilution factor of about 0.50. At each cycle 500 ml test solution or control water was delivered to each flow-splitting dilution chamber. These chambers, which were randomly positioned on the diluter, diverted ca. 125 ml to each of four replicate test chambers at each test concentration and the control during the acute test. During the chronic study, the 125 ml delivered from the Splitter cells to each replicate was split five ways into each of the five tubes contained within a replicate beaker. The diluter was set to cycle every 30 minutes resulting in a minimum of 15 volume replacements in each beaker per day.

Statistics: The LC50 and 95% confidence intervals were determined for the 48-hour acute test using probit analysis. The LC50 values were based on analyzed concentrations. The LC50 value is the statistically determined concentration of the test material at which 50% of the test organisms would die within a specified time interval.

Result :

The mean biphenyl concentrations derived from the analyzed test solutions during the acute test are shown in the table. All analyzed concentrations were within a range of 63.3 to 97.6% of nominal. The calculated 48-hr LC50 value for biphenyl was 0.36 mg/L (95% confidence interval: 0.28 to 0.47 mg/L). The no observable effect level was 0.04 mg/L and the 100 % kill concentration was > 0.96 mg/L. There was no mortality in the acetone controls and 1%, mortality in the water controls over the 48 h test period. No sublethal effects were observed during this test.- The dissolved oxygen (D.O.) measurements throughout the test were all >90% saturation. The pH and temperature measurements ranged from 7.4 to 7.9 and 20.5 to 20.7°C, respectively.

Analy Conc (mg/L)	No Daphnia	% Dead 24-hr	% Dead 48-hr
0.96	30	30	87
0.48	30	7	57
0.24	30	0	40
0.09	30	0	7
0.04	30	0	0
Acetone	30	0	0
Water	30	0	3

No sublethal effects were noted during the test.

Test condition :

Conditions	
Temperature	20 ± 1°C
Photoperiod	16 hrs light/8 dark
Daphnid source	laboratory reared
Diet	NA
Test Vessel	400 mL beaker
Observations	D.O. pH,temperature, mortality 0, 24, 48 hrs
Effect Criteria	mortality-immobility
Length of Test	48 hrs

Analytically determined concentrations

Nominal Concen. (mg/L)	Day 0 (mg/L)	24 H (mg/L)	48 H (mg/L)	Mean ± S. D. (n = 6)	Percent Nominal
1	1.13	0.797	0.965	0.964 ±0.161	96.4
0.5	0.529	0.433	0.493	0.485 ±0.048	97
0.25	0.258	0.215	0.259	0.244 ±0.025	97.6
0.13	0.103	0.086	0.093	0.094 ±0.009	72.3
0.06	0.043	0.034	0.037	0.038 ±0.005	63.3
Acetone	N.D.	N.D.	N.D.	N.D.	
Water	N.D.	N.D.	N.D.	N.D.	

Water Quality Measurements

Temperature Range:	20.5 to 20.7 °C
pH Range:	7.4 to 7.9
Dissolved Oxygen:	> 90% saturation.

Test substance :

Biphenyl, purity > 99.2% CASNO 92-52-4

Conclusion :

-The 24-hour EC50 for biphenyl under these conditions is 1.3 mg/L (95% confidence limits of 1.0 - 3.9)

-The 24-hour NOEC for biphenyl under these conditions is 0.24 mg/L

-The 48-hour EC50 for biphenyl under these conditions is 0.36 mg/L (95% confidence limits of 0.28 - 0.47)

-The 48-hour NOEC for biphenyl under these conditions is 0.04 mg/L

No sublethal effects were noted during the test

Reliability : (1) valid without restriction

High quality guideline-like study under GLP with analytical support

Flag : Critical study for SIDS endpoint

14.12.2003 (15)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= .25 measured/nominal
EC50	:	= .73 measured/nominal
Limit Test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method :

A static toxicity test was conducted using clear-capped glass jars (8 oz) containing about 200 mL test solution. The dilution water was local well water. The test substance, dissolved in dimethyformamide (DMF), was pipetted into 1000 ml of dilution water and shaken for 1 minute for each concentration. This solution was then divided into three 200 ml aliquots in triplicate jars. The remaining 400 mL were used for 0-hour DO, pH, alkalinity and hardness determinations. A control, consisting of the same dilution water and conditions but without test material was established. Also, a solvent control was employed which consisted of dilution water and the maximum amount of solvent used in the test concentrations (0.5 ml/L). Special attention was given to place polyethylene lined caps on all jars after the Daphnia were added to prevent any loss of Biphenyl due to volatilization. The caps were removed only once during the study to count the Daphnia at 24 hours

All test vessels were maintained at room temperature without aeration during the test. Ten daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 30 daphnids per concentration level and controls. During this test, the dissolved oxygen concentration, pH, alkalinity, hardness, conductivity and temperature of test solutions were monitored at the initiation in the control and high text concentration and termination of the toxicity test in the high, middle, low and control test concentrations.

Statistical methods:
Test concentrations and corresponding percent immobilization data derived from definitive tests were used to calculate the 48-hour median effect concentration, EC50, and 95% confidence intervals.
In tests where the highest percentage immobilization was > 65 percent, the computer program of Stephan, which calculates EC50 by three methods, binomial, moving average, and probit analysis, was used (Stephan). For tests in which the immobilization did not exceed 50 percent, the EC50 is reported as greater than the highest test concentration. If the highest percentage immobilization was >50 <65 percent, the EC50 is estimated by the program of Stephan and is reported as an estimate.

Stephan, C. E. 1976. Methods for Calculating an LC50. In Aquatic Toxicology and Hazard Evaluation, F. L. Mayer and J. L. Hamelink Editors.

Remark :

The fact that it was stated in the report that the water solubility of the test material was exceeded at 2.0 mg/L and above is of concern. The published value for water solubility of biphenyl is 7.38 mg/L. It is possible the water

conditions were such that the solubility was reduced; however, not considered likely based on the study reported in 1983 using essentialy the same conditions in which the test material was reported to be fully soluble up to 5 mg/L

Result :

Visual inspection of the beakers indicated that the water solubility was exceeded at concentrations of 2.0 mg/L and higher. This should not effect the EC50 valuesince the key data points used to calculate the 48-hour EC50 were derived from exposure concentrations which were in solution.

Concentrations tested and corresponding percent immobilization of Daphnia magna exposed to Biphenyl.

	PERCENT IMMOBILIZATION	
CONC mg/L	24-Hrs	48-Hrs
Control	0	0
Solvent Control	0	0
0.25	0	3.3
0.50	0	13.3
1.0	3.3	76.6
2.0	0	100
4.0	26.6	100

Test condition :

During the 48-hour toxicity test with Biphenyl, the pH and dissolved oxygen ranged from.7.9 to 8.2 and 7.0 to 8.6 mg/L, respectively (Tables 2 and 3 and Appendix I). The average temperature was 22°C and the alkalinity and hardness ranged from 220 to 258 mg/L and 230 to 262 mg/L.

PARAMETER	CONC. (mg/L)	TIME 0-Hr	48-Hr
Temperature (°C)	Control	22	22
	0.25	22	22
	1	22	22
	4	22	22
D.O. (mg/L)	Control	7.5	8
	0.25	7	8.3
	1	7.9	8.7
	4	7.6	8.6
pH	Control	8	7.9
	0.25	8.2	8
	1	8.2	8
	4	8	7.9
Alkalinity (mg/L)	Control	232	238
	0.25	230	258
	1	242	220
	4	226	250
Hardness (mg/L)	Control	256	230
	0.25	274	250
	1	230	246
	4	262	236

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Under these conditions, the 48-hour EC50 for Daphnia magna is 0.73 mg/L (confidence limits of 0.63 to 0.85) and the NOEC is 0.25 mg/L

Reliability : (2) valid with restrictions

Although this study was not conducted under full GLPs, it was conducted by a scientifically defensible method following a standard laboratory guideline it was stated that the water solubility of the test material was exceeded at 2.0 mg/L and above.

14.12.2003 (3)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= 1.8 measured/nominal
EC50	:	= 3.65 measured/nominal
Limit Test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method :

A static toxicity test was conducted using clear-capped glass jars (8 oz) containing about 200 mL test solution. The dilution water was local well water. The test substance, dissolved in dimethyformamide (DMF), was pipetted into 1000 ml of dilution water and shaken for 1 minute for each concentration. This solution was then divided into three 200 ml aliquots in triplicate jars. The remaining 400 mL were used for 0-hour DO, pH, alkalinity and hardness determinations. A control, consisting of the same dilution water and conditions but without test material was established. Also, a solvent control was employed which consisted of dilution water and the maximum amount of solvent used in the test concentrations (0.5 ml/L).

Nominal test concentrations were selected based on a rangefinding test. All test vessels were maintained at room temperature without aeration during the test. Ten daphnids were randomly assigned to each test vessel within 30 minutes after the compound was added for a total of 30 daphnids per concentration level and controls. During this test, the dissolved oxygen concentration, pH, alkalinity, hardness, conductivity and temperature of test solutions were monitored at the initiation in the control and high text concentration and termination of the toxicity test in the high, middle, low and control test concentrations.

Statistical methods:
Test concentrations and corresponding percent immobilization data derived from definitive tests were used to calculate the 48-hour median effect concentration, EC50, and 95% confidence intervals.
In tests where the highest percentage immobilization was > 65 percent, the computer program of Stephan, which calculates EC50 by three methods, binomial, moving average, and probit analysis, was used (Stephan). For tests in which the immobilization did not exceed 50 percent, the EC50 is reported as greater than the highest test concentration. If the highest percentage immobilization was >50 <65 percent, the EC50 is estimated by the program of Stephan and is reported as an estimate.

Stephan, C. E. 1976. Methods for Calculating an LC50. In Aquatic Toxicology and Hazard Evaluation, F. L. Mayer and J. L. Hamelink Editors.

Result :

A summary of the percent immobilization during this study is presented in the table below.

Visual inspection of the beakers indicated that the water solubility was not exceeded at any concentration.

CONC mg/L	PERCENT IMMOBILIZATION 24-Hrs	48-Hrs
Control	0	0
Solvent Control	0	0
0.65	0	0
1.08	0	0
1.8	0	0
3	0	17
5	0	97

Concentrations tested and corresponding percent immobilization of Daphnia magna exposed to Biphenyl.

Test condition :

The pH and dissolved oxygen ranged from 7.9 to 8.0 and 8.0 to 8.8 mg/L, respectively. The mean temperature was 22.2 deg C and the alkalinity and hardness ranged from 250 to 266 mg/L and 242 to 248 mg/L.

PARAMETER	CONC. (mg/L)	T I M E 0-Hr	48-Hr
Temperature (°C)	Control	21.7	22.7
DO (mg/L)	Control	8.5	8.5
	0.65		8.8
	1.8		8.6
	5	8	8.4
pH	Control	7.9	8
	0.65		8
	1.8		8
	5	7.9	8
Alkalinity (mg/L)	Control	250	266
	0.65		260
	1.8		254
	5	256	250
Hardness (mg/L)	Control	246	242
	0.65		246
	1.8		242
	5	248	244
Conductivity	Control	800	700
	0.65		800
	1.8		725
	5	800	750

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Under these conditions, the 48-hour EC50 for Daphnia magna is 3.65 mg/L (confidence limits of 3.24 to 3.93) and the NOEC is 1.8 mg/L

Reliability : (2) valid with restrictions

Although this study was not condusted under full GLPs, it was conducted by a scientifically defensible method following a standard laboratory guideline and is considered to have high reliability.

13.12.2003 (4)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Chlamydomonas sp. (Algae)
Endpoint	:	growth rate
Exposure period	:	3 hour(s)
Unit	:	mg/l
EC50	:	= 1.3 measured/nominal
Limit test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method :

Cultures of the green alga Chlamydomonas angulosa (#680 Indiana collection) were grown under axenic conditions using Bold's Basal medium (BBM) in cotton-plugged 125 m1 Erlenmeyer flasks. The media was at a pH of 6.5 and a 12-hour light-dark cycle was used with a light intensity of 400 foot candles, and a temperature of 19°C.

Saturated solutions were prepared by stirring the solid biphenyl in sterile BBM for 24 hours. The saturated solution was then decanted or filtered, and dilutions made with BBM to provide 0, 20, 50 and 100 percent of the original saturation level of biphenyl.

Radiolabeled carbon dioxide (carbon 14) uptake was used as a measure of photosynthesis. Radiolabel was added to provide an activity of 1.25 pCi/100 ml.

Three-to-four day exponential phase cells were used for experiments, at a cell concentration of 5 x 104 cells/ml of C. angulosa. Labeled carbon dioxide was added at time zero, the flasks were sealed with a glass stopper and were incubated under the described conditions for three hours. A dark set of controls was also run. After filtering, the cells were washed with 0.85 percent saline to remove surface sodium bicarbonate and radioactivity was determined on dried Millipore filters using an end window radiation detector (Nuclear Chicago).

Although this determination of growth inhibition was of short duration, sensitive and precise measures of growth were employed that are considered to provide an accurate estimate of the IC50. In addition, the high volatility of biphenyl from open aqueous systems (Henry's Law constant 2.5 x 10-4 atm-m3/mol) indicates that loss of test material over a period of hours will be significant. (A volatilization half-life of 4.3 hours was estimated for biphenyl in a stream 1 m deep, flowing 1 m/second, with an air current of 3 meters/second*). Thus, studies in open systems of duration longer than a few hours are not anticipated to be robust tests of inhibition due to the constant and rapid reduction of test material

concentration.

* USEPA. Health and Environmental Effects Profile for 1,1'-biphenyl. Environmental Criteria and Assessment Office, Cincinnati, OH, 35 pp 1984.

Result :

Percent inhibition of radiocarbon uptake at each dilution 0, 20, 50 or 100% of saturation was plotted and the concentration that caused a 50% inhibition (1.3 mg/L) was determined. Thirty-eight different hydrocarbons were tested using essentially the same procedure and the IC50 values were plotted versus both water solubility and Kow (log-log plot). A regression line was drawn through the data and the fit was found to be good with a correlation coefficient between 0.80 and 0.93. Data from biphenyl fell very close to the regression line indicating high precision for the estimate of IC50 for biphenyl.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The 3-hour IC50 for growth of the green alga Chlamydomonas angulosa was determined to be 1.3 mg/L under these conditions.

Reliability : (2) valid with restrictions

Well-documented published study using a sensitive and precise means of measuring algae growth inhibition.

Flag : Critical study for SIDS endpoint

18.12.2003 (18)

Species : Chlorella vulgaris (Algae)
Endpoint : growth rate
Exposure period : 3 hour(s)
Unit : mg/l
EC50 : = 3.9 measured/nominal
Method :
Year :
GLP : no data
Test substance :

Method :

Cultures of the green alga Chlorella vulgaris (#260 Indiana collection) were grown under axenic conditions using Bold's Basal medium (BBM) in cotton-plugged 125 m1 Erlenmeyer flasks. The media was at a pH of 6.5 and a 12-hour light-dark cycle was used with a light intensity of 400 foot candles, and a temperature of 19°C.

Saturated solutions were prepared by stirring the solid biphenyl in sterile BBM for 24 hours. The saturated solution was then decanted or filtered, and dilutions made with BBM to provide 0, 20, 50 and 100 percent of the original saturation level of biphenyl.

Radiolabeled carbon dioxide (carbon 14) uptake was used as a measure of photosynthesis. Radiolabel was added to provide an activity of 0.5 p Ci/100 ml.

Three-to-four day exponential phase alga cells were used for experiments,

at a cell concentration of 20 x 104 cells/ml. Labeled carbon dioxide was added at time zero, the flasks were sealed with a glass stopper and were incubated under the described conditions for three hours. A dark set of controls was also run. After filtering, the cells were washed with 0.85 percent saline to remove surface sodium bicarbonate and radioactivity was determined on dried Millipore filters using an end window radiation detector (Nuclear Chicago).

Remark :

Although this determination of inhibition was of a short duration, sensitive and precise measures of growth were employed that are considered to provide an accurate estimate of the IC50. In addition, the high volatility of biphenyl from open aqueous systems (Henry's Law constant 2.5 x 10-4 atm-m3/mol) indicates that loss of test material over a period of hours will be significant. (A volatilization half-life of 4.3 hours was estimated for biphenyl in a stream 1 m deep, flowing 1 m/second, with an air current of 3 meters/second*). Thus, studies in open systems of duration longer than a few hours are not anticipated to be robust tests of inhibition due to the constant and rapid reduction of test material concentration.

* USEPA. Health and Environmental Effects Profile for 1,1'-biphenyl. Environmental Criteria and Assessment Office, Cincinnati, OH, 35 pp 1984.

Result :

Percent inhibition of radiocarbon uptake at each dilution 0, 20, 50 or 100% of saturation was plotted and the concentration that caused a 50% inhibition (3.9 mg/L) was determined. Thirty-eight different hydrocarbons were tested using essentially the same procedure and the IC50 values were plotted versus both water solubility and Kow (log-log plot). A regression line was drawn through the data and the fit was found to be good with a correlation coefficient between 0.80 and 0.93. Data from biphenyl fell precisely on the regression line indicating high precision for this IC50 estimate.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The 3-hour IC50 for growth of the green alga Chlorella vulgaris was determined to be 3.9 mg/L under these conditions.

Reliability : (2) valid with restrictions

Well-documented published study using a sensitive and precise means of measuring algae growth inhibition.

18.12.2003 (18)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 2400 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals :
Vehicle : other: corn oil
Doses : 2000, 2510, 3160 or 3980 mg/kg bw

Method :

The undiluted test substance was administered as a 20% solution in corn oil to Sprague-Dawley rats at four dose levels using two or three animals of each sex per group. Treated animals were observed for 14 days, survivors were sacrificed and subjected to an examination of the viscera. Body weights were only reported at the time of dosing (presumably used to determine the volume of test material to administer). Rats of each sex were used and all were in an initial weight range of 210 to 235 grams. Dose levels and animals per group are given in the results.

Result :

Dose levels, grouping and mortality were as follows:

	MORTALITY	
Dose(mg/kg)	Males	Females
2,000	1/3	0/2
2,510	1/2	2/3
3,160	1/3	2/2
3,980	2/2	3/3

Deaths occurred one to five days after dosing with most occurring within two days. Clinical signs reported were loss of appetite and activity for two to six days following administration and for moribund animals, increasing weakness, ocular discharge, collapse and death.

Necropsy revealed hemorrhagic areas of the lungs, slight discoloration of the liver and gastrointestinal inflammation.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The oral LD50 is 2,400 mg/kg with a 95% confidence limit of 2,180 to 2,640 mg/kg in Sprague-Dawley rats of combined sex.

Reliability : (2) valid with restrictions

Good documentation for an older study. Considered reliable but downgraded to 2 due to lack of individual animal data.

Flag : Critical study for SIDS endpoint

02.11.2003 (25)

Type	:	LD50
Value	:	= 3280 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	no data
Number of animals	:	60
Vehicle	:	other: olive oil
Doses	:	

Method :

Sprague-Dawley rats were administered purified Biphenyl as a 25% solution in olive oil by gavage. Group size and dose levels were not specified except that it was reported that 60 rats were utilized to determine the oral LD50 of Biphenyl in the rat. Six other materials were also reported on in the publication. After dosing rats were observed for adverse clinical signs. It is not stated how long the observation period was after dosing. It is stated that one rat exposed orally to Biphenyl died 2 days after dosing and it is noted that some animals in the larger study survived up to 18 days after dosing. It is, therefore reasonable that the post-dosing observation was 18-days. Evidence that necropsy examinations were performed comes from statements concerning the local effects of Biphenyl on the GI tract.

Result :

The purified-Biphenyl oral LD50 for rats is listed as 3.28 g/kg in a table. It is also listed that 60 rats were used to make this determination and the survival time varied from 18 hours to 2-days. Dose levels and mortalities are not provided. Clinical signs of toxicity are noted generally for all compounds as "inducing a state of intoxication characterized by an increased respiratory rate. Lacrimation, loss of appetite, loss of body weight, muscular weakness, unsteadiness and respiratory difficulties, and terminated by death in coma." Other compounds that were studied are o and p-aminodiphenyl, o and p-nitrodiphenyl and dihydroxyoctachlorodiphenyl.

Necropsy results from animals dying on test are generically described as it caused little or no local injury, except for slight irritative effects in the stomach, duodenum and upper jejunum of animals that died within a few hours after dosing.
The report mentions that effects on the kidneys and liver were observed but the report does not indicate if there were from Biphenyl or other Biphenyl derivatives that were dosed. Likewise slight to to severe toxic degenerative changes in the myocardium were reported but it is not clear if there were associated with Biphenyl of the other compounds.

Test substance :

Biphenyl, purified. CASNO 92-52-4

Conclusion :

The oral LD50 of Biphenyl in the Sprague-Dawley rat is 3280 mg/kg body weight.

Reliability : (2) valid with restrictions

Published reports are assigned a reliability of 2. Despite differences from the current guideline and the lack of details that would be reported in a modern investigation, the study appears to have been well conducted. This sudy also uses more animals than other determinations of LD50 for this material and may be the most accurate determination of the LD50.

02.11.2003 (14)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	
Species	:	rat
Strain	:	other: CFE
Sex	:	female
Number of animals	:	6
Vehicle	:	other: air
Doses	:	saturation
Exposure time	:	8 hour(s)

Method :

A group of 6 female CFE albino rats weighing 126 to 131 grams were exposed for 8 hours to vapors, mists and decomposition products of Biphenyl produced by passing air at a rate of 2.5 L/min through a fritted glass disk immersed one inch into 50 ml heated Biphenyl in a bubbler, which was in turn submerged in a silicone bath at 176 deg C. The inhalation chamber was 9-L in volume. Liquefied Biphenyl in the bubbler never exceeded 166 deg C in temperature and the air temperature in the chamber averaged about 27 deg C. Animals were observed for 14 days after exposure.

Result :

No animals died during the exposure or subsequent observation period. All animals gained weight (50 to 65 grams) during the observation period and no gross pathology was found at sacrifice.

Test substance :

Biphenyl, purified, ca. 99%. CASNO 92-52-4

Conclusion :

Inhalation of saturated vapors of purified (ca 99%) biphenyl for 8-hours did not produce any mortality in female rats.

Reliability : (2) valid with restrictions

Good documentation for an older study. Considered reliable but downgraded to 2 due to lack of details, including measurement of concentration and details of clinical observations.

02.11.2003 (23)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 5010 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: Corn oil
Doses	:	

Method :

The test substance was administered as a 40% solution/suspension in corn oil to the closely-clipped skin of New Zealand white male or female rabbits weighing 2.0 to 2.2 kg at dosing. The exposure period is listed as 24 hours and it was typical at that laboratory to cover the exposed skin with plastic that was held in place for 24 hours but the exact conditions are not

specified. Animals were observed for 14 days after treatment, sacrificed and necropsied. Increasing incremental doses were used to minimize animal usage.

Result :

Dose levels and grouping were as follows:

Dose (mg/kg)	Animals	Result
5,010	1 F	no deaths
7,940	1M&1F	male died

Clinical signs reported were loss of appetite and activity for two to three days following administration in survivors and increasing weakness, collapse and death for the decedent. Necropsy of the animal that died indicated lung and liver hyperemia, slightly enlarged gall bladder and gastrointestinal inflammation

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The Dermal LD50 is greater than 5010 mg/kg in New-Zealand rabbits.

Reliability : (2) valid with restrictions

Good documentation for an older study. This study is considered an adequate test of approximate dermal toxicity. Procedure is similar to current OECD-423 Acute Toxic Class Method. Only study available that used appropriate vehicle.

Flag : Critical study for SIDS endpoint

06.11.2003 (25)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type : Chronic
Species : rat
Sex :
Strain :
Route of admin. : oral feed
Exposure period : 750 days
Frequency of treatm. : Constant
Post exposure period :
Doses : 10, 50, 100, 500, 1000, 5000 or 1000 ppm
Control group : yes, concurrent vehicle
Method :
Year :
GLP : no
Test substance :

Method :

Groups of 15 weanling rats of each sex were placed on diets containing seven levels of biphenyl for a period of 750 days. Animals were housed 5 to a cage and had free access to food and water at all times. During the period of growth, rats were weighed once a week and food consumption was determined weekly. Following the period of active growth, the rats were weighed at 50-day intervals for the duration of the study. Animals

were examined at the time of weighing for gross evidence of tumors. At sacrifice, animals were necropsied, weights of liver, kidneys, heart, and testes were determined. Hematoxylin-eosin stained sections of heart, lung, liver, kidney, adrenal, spleen. pancreas, stomach, intestine, bladder, thyroid, brain, pituitary, and gonads were prepared and bone marrow smears of representative animals were prepared.

Dosed feed levels for the study were (in ppm) 0, 10, 50, 100, 500, 1000, 5000 or 10000 ppm (0.001 to 1%).

Studies on possible reproductive effects and survival of young were also conducted as follows. Ten weanling female and five males rats were placed on control diet for 60 days, and subsequently mated, one male to two females. An identical experiment included Biphenyl at a dietary level of 0.1%. Nine female and 3 male rats were fed a dietary level of 0.5% Biphenyl in a subsequent study. All rats continued exposure until the pups of all litters were weaned.

In a second series of experiments, 90-day old rates were exposed for 11 days before mating and continuously until weaning of pups. Using this dosing schedule, 8 female and 4 male rats were placed on the control diet, 8 females and 4 males received 0.1%, and 9 females and 3 males received 0.5% dietary levels of Biphenyl.

Result :

Survival of animals was only reduced at the two highest concentrations. Details are shown in the table below.

Number of surviving animals/group-time:

		Days on Test										
	ppm	0	50	100	150	200	250	300	350	450	550	650	750
Male	0	15	15	14	14	14	14	14	13	12	12	10	9
	10	15	15	15	14	14	14	14	12	12	12	12	8
	50	15	15	15	15	15	15	15	14	14	14	13	10
	100	15	14	14	14	14	13	13	12	12	12	12	11
	500	15	15	14	14	14	14	14	14	14	14	14	13
	1,000	15	15	15	15	15	15	15	12	11	10	10	10
	5,000	15	15	14	14	14	14	14	11	9	9	5	2
	10,000	15	14	14	14	13	13	12	11	10	8	5	2
Female	0	15	15	15	15	15	15	15	15	15	13	12	9
	10	15	15	15	15	15	15	15	15	14	13	8	6
	50	15	13	13	12	12	12	12	11	10	10	6	5
	100	15	15	15	15	15	15	15	14	13	12	11	11
	500	15	15	15	15	15	15	15	12	11	11	1	5
	1,000	15	15	15	15	14	13	13	9	9	7	7	5
	5,000	15	15	15	14	14	14	13	11	11	9	6	5
	10,000	15	15	14	14	13	13	11	9	7	5	3	2

Body weight gain was reduced for the top two concentration groups

MALE BODY WEIGHTS (group mean)

	Days on Test											
Feed	0	50	100	150	200	250	300	350	450	550	650	750
0	41	247	331	372	386	397	418	435	449	447	449	401
10	41	228	309	357	374	390	406	411	437	449	444	423
50	41	227	303	355	368	376	390	403	418	427	425	378
100	41	236	311	355	370	383	405	423	431	443	433	428
500	42	239	316	357	365	376	387	404	406	410	396	390
1,000	42	239	322	352	366	367	378	395	406	413	407	393
5,000	42	193	261	310	303	320	326	337	322	332	369	367
10,000	42	143	199	223	248	252	260	272	285	286	228	-

FEMALE BODY WEIGHTS (group mean)

	Days on Test											
Feed	0	50	100	150	200	250	300	350	450	550	650	750
0	41	167	210	244	259	281	287	299	337	360	366	328
10	41	170	217	253	267	282	292	298	321	340	345	375
50	41	162	209	247	260	277	282	294	310	339	356	348
100	41	169	208	244	260	278	283	293	323	348	397	357
500	41	182	210	235	246	260	261	264	300	312	321	313
1,000	40	162	207	235	252	257	252	253	304	337	343	332
5,000	41	143	180	205	218	230	229	239	258	261	257	236
10,000	41	131	152	169	177	187	187	195	202	195	188	-

Organ weights of treated animals at sacrifice were similar to controls except the highest concentration was associated with increased relative kidney weights.

Biphenyl Conc	# rats	Body wt (g)	Mean organ weights /100 g body weight (grams)			
Males			Liver	Kidneys	Heart	Testes
0	9	396±24.6	2.89±0.16	0.75±0.02	0.32±0.015	0.72±0.03
10	8	424±5.1	2.66±0.06	0.70±0.03	0.28±0.008	0.62±0.07
50	10	383±19.8	2.84±0.15	0.73±0.02	0.30±0.01	0.56±0.06
100	11	394±14.2	2.47±0.07	0.72±0.01	0.31±0.008	0.67±0.07
500	13	371±15.8	3.03±0.12	0.74±0.02	0.31±0.007	0.65±0.06
1,000	10	366±23.7	2.98±0.19	0.83±0.05	0.34±0.012	0.60±0.08
5,000	2	345	3.12	1.17	0.36	0.36
Females						
0	9	333±9.4	3.11±0.15	0.65±0.01	0.33±0.01	
10	6	369±13.4	3.21±0.17	0.62±0.02	0.28±0.07	
50	5	335±16.6	2.81±0.18	0.64±0.02	0.31±0.03	
100	11	341±9.1	3.46±0.74	0.62±0.02	0.30±0.01	
500	5	306±12.5	3.51±0.12	0.68±0.02	0.31±0.01	
1,000	5	327±6.8	3.18±0.10	0.65±0.01	0.32±0.01	
5,000	5	226±25.8	4.52±0.20	1.39±0.14	0.46±0.04	

HISTOPATHOLOGICAL FINDINGS: The only histopathological change that was clearly related to biphenyl consumption occurred in the kidneys. The kidneys of all male and female rats receiving dietary levels of 0.5 or 1.0% biphenyl had prominent irregular scarring, lymphocytic infiltration, tubular atrophy, and patchy tubular dilation to the point of cyst formation. Hemorrhage was present in some dilated tubules and, in some instances, in the renal pelvis. Calculi with basophilic staining foci were frequent it the renal pelvis and similar smaller deposits of precipitated material were sometimes seen in the kidney substance. Some of the dilated tubules contained polymorphonuclear leucocytes and small fragments of nuclear material. Hydronephrosis was common and in several instances there was

metaplasia of the epithelium of the renal pelvis to the squamous cell type, but this did not appear to be neoplastic.

Kidneys of female rats on doses of 0.1% or less Biphenyl exhibited no changes that were clearly different from the occasional small scars and focally dilated tubules that were present in the control animals.

In the kidneys from the male animals at all dose levels including the controls, scars and dilated tubules were distinctly more numerous and some degree of hydronephrosis more prominent than in the females. This corresponded to the observation of deposited material in the renal pelvis or bladder in male animals only, except at the 0.5 and 1.0% feed levels where it was also present in female rats. Most of the kidneys from male rats which received 0.1% or 0.03% Biphenyl similar to controls, except in two of these animals there were masses of partly disintegrated blood in the rectal pelvis and in two others, there were small basophilic concretions in the medullary portions of the kidneys.

Blood was present in the renal pelvis in one animal from each of the other treated groups (0.01, 0.005 and 0.001%). These deposits were sometimes associated with hydronephrosis. Hydronephrosis was also present in several kidneys from the other groups of male animals (including controls) in which pelvic hemorrhage or concretions were not demonstrable. Some of these animals, as well as some others without observed hydronephrosis, presented a protein coagulum in their bladder. This was present in several of the control animals and was clearly unrelated to the treatment. There was a small amount of old blood in the pelvis of one control kidney.

Comparison of the kidneys from the various groups of animals indicated that with doses of 0.1% or less Biphenyl there was no distinct difference from the controls.

No other organ changes could be related to biphenyl ingestion.

PAIRED FEEDING: As records of food consumption revealed a decrease, paired feeling experiments with rats of each sex receiving 1.0 and 0.5% biphenyl were conducted for 98 days to determine whether the decrease in growth could be accounted for by reduced food consumption. Thirty-eight males and 46 females were used in this paired-feeding experiment. Six weanling rats of each sex were used as ad libitum food consumption controls, 9 males and 10 females were pair fed based on food consumption at 0.5% Biphenyl, and 7 males and 10 females were pair fed at the 1.0% Biphenyl food consumption level. The mean body weights at the end of the 98-day pair feeding study were for MALES: ad lib 0% 232 g, ad lib 0.5% 203 g, ad lib 1.0% 172 g; pair-fed control diet at food consumption rate of 0.5% animals, 199g; pair-fed control diet at food consumption rate of 1.0% animals,170g. FEMALES ad lib 0% 150 g, ad lib 0.5% 126 g, ad lib 1.0% 113 g; pair-fed control diet at food consumption rate of 0.5% animals, 123 g; pair-fed control diet at food consumption rate of 1.0% animals,107g. This indicates that reduced feed consumption and not toxicity was probably responsible for much of the reduced weight gain associated with the groups fed Biphenyl in their diet.

Not shown are reductions in hemoblogin levels measured after 300 days of dosing in the two highest concentrations of dietary biphenyl. Hemoglobin levels were reduced about 30% in the highest concentration but this was attributed to the reduced feed consumption and reduction in weight gain

and not a direct effect of the chemical on blood or blood-forming organs.

The incidence of tumors was examined as a function of test substance administration. The incidence of all tumors in treated groups was similar to controls. The number of animals on test, however, would not provide a sensitive bioassay for carcinogenicity.

REPRODUCTIVE TOXICITY TEST:

Two studies of potential reproductive effects and survival of young were conducted. It was concluded that "Dietary levels of 0.1 and 0.5% Biphenyl had no significant effect on reproduction." Please refer to the reproductive toxicity section of this HPV document for details.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Feeding dietary levels of 5,000 or 10,000 ppm Biphenyl to rats for up to 750 days was associated with a decrease in weight gain and pathological changes in the kidneys. The reduced body weight gain was attributed to lack of palatability and not a toxic effect of biphenyl. A dietary level of 1000 ppm is considered a NOAEL. In the limited reproductive toxicity test, no effect on reproductive ability or pup survival was found.

Reliability : (2) valid with restrictions

Published reports are assigned a reliability of 2. Despite differences from current protocols, this was a well documented study of considerable scope.

Flag : Critical study for SIDS endpoint

14.12.2003 (5)

Type	:	Chronic
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344/DuCrj
Route of admin.	:	oral feed
Exposure period	:	104 weeks
Frequency of treatm.	:	cont
Post exposure period	:	none
Doses	:	500, 1500, 4500 ppm
Control group	:	yes, concurrent vehicle
LOAEL	:	= 500 ppm
Method	:	
Year	:	
GLP	:	yes
Test substance	:	

Method :

A chronic study using F344/DuCrj rats, performed according to standard protocols, showed a significant increase in neoplastic and non-neoplastic lesions of the urinary bladder and, in high-dose males, a significant increase in calculi within the urinary bladder. In this 104 week study, dietary concentrations of Biphenyl were 0, 500, 1500, or 4500 mg/kg-day (0, 38, 113, or 338 mg/kg body weight per day).

The study report was not available for review; this information is excerpted from the IPCS CICAD (#6) Biphenyl 1999.

Result :

A chronic study using F344/DuCrj rats, performed according to standard protocols, showed a significant increase in neoplastic and non-neoplastic lesions of the urinary bladder and, in high-dose males, a significant increase in calculi within the urinary bladder. In this 104 week study, dietary concentrations of Biphenyl were 0, 500, 1500, or 4500 mg/kg-day (0, 38, 113, or 338 mg/kg body weight per day).

The study report was not available for review, this information is excerpted from the IPCS CICAD (#6) Biphenyl 1999.

A dose-dependent increase in hyperplasia of the renal pelvis epithelium was reported. Histopathological findings for the kidneys and urinary bladder are summarized in the following table. Other findings included increased serum levels of alkaline phosphatase, aspartate transaminase, and alanine transaminase and an increased urea nitrogen level in low-dose males and mid-dose females, which became more pronounced with increasing doses. Hematological effects were reported in mid- and high-dose females and in high-dose males. A LOEL of 38 mg/kg was derived from this study (it is not clear if this LOEL was assigned by IPCS/WHO or by the report authors).

Summary of Effects:

High dose: Transitional cell carcinoma in males, bladder hyperplasia in males and females, kidney hyperplasia and mineralization in males and females, clinical chemical and hematological changes in males and females, increase in urea nitrogen in males and females.

Mid dose: Kidney mineralization in males and females (minimal), increase in urea nitrogen (males and females), clinical chemical and hematological changes (males and females).

Low dose: Increase in urea nitrogen (males), clinical chemical and hematological changes (males)

	Of 50 Males				Of 50 Females			
End-point	0 mg/kg	500 mg/kg	1500 mg/kg	4500 mg/kg	0 mg/kg	500 mg/kg	1500 mg/kg	4500 mg/kg
Survival (of 50)	37	41	38	31	44	38	44	37
-URINARY BLADDER (Neoplastic)								
transitional cell papilloma	0	0	0	10	0	0	0	0
transitional cell carcinoma	0	0	0	24*	0	0	0	0
squamous cell papilloma	0	0	0	1	0	0	0	0
squamous cell carcinoma	0	0	0	1	0	0	0	0
-TRANSITIONAL EPITHELIUM								
simple hyperplasia	0	0	0	12	0	0	1	1
nodular hyperplasia	0	0	0	40	1	0	0	5
papillary hyperplasia	0	0	0	17	0	0	0	4
basal cell hyperplasia	0	0	0	27	0	0	0	4
squamous cell hyperplasia	0	0	0	13	0	0	0	1
squamous cell metaplasia	0	0	0	19	0	0	0	4
inflammatory polyps	0	0	0	10	0	0	0	0
calculi	0	0	0	43	0	0	0	8

-KIDNEY LESIONS								
mineralization -- papilla	9	9	14	23	2	6	3	13
mineralization -- pelvis	9	6	10	18	12	12	18	27
calculi	0	0	0	13	0	0	0	3
desquamation -- pelvis	1	0	0	11	0	0	0	2
simple hyperplasia of the transitional epithelium	6	8	5	19	3	5	12	25
nodular hyperplasia of the transitional epithelium	0	1	1	21	0	0	1	12
ureter dilatation	0	0	1	14	0	0	0	6

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The kidney is the primary target organ and the low-dose, 500 ppm, is considered a LOEL based on BUN and clinical chemistry, the low-dose, 500 ppm, is a NOAEL based on histopathology.

Reliability : (2) valid with restrictions

A modern guldeline-like study, assignes a 2 because the report was not available for review.

14.12.2003 (20)

Type : Chronic
Species : mouse
Sex : male/female
Strain : other: Crj:BDF1
Route of admin. : oral feed
Exposure period : 104 weeks
Frequency of treatm. : continuous
Post exposure period : none
Doses : 100, 300, 900 mg/kg-day
Control group : yes, concurrent vehicle
Method :
Year :
GLP : yes
Test substance :

Method :

A chronic study using Crj:BDF1 mice of each sex was performed according to standard protocols. Groups of 50 mice of each sex were given diets containing 0, 667, 2000, or 6000 mg biphenyl/kg (0, 100, 300, or 900 mg/kg body weight per day) for 104 weeks. At the end of the dosing period surviving mice were sacrificed, examined for gross effects, tissues were removed, fixed, sliced, stained with H&E and examined for microscopic changes/

The study report was not available for review; this information is excerpted from the IPCS CICAD (#6) Biphenyl 1999.

Result :

After 104 weeks of biphenyl administration, a slight increase in liver tumors (hepatocellular adenomas and carcinomas) and basophilic cell foci of the liver was observed in the females at doses of 300 and 900 mg/kg body weight per day; however, the effects were not concentration dependent and the statistical significance was marginal, as shown in the tables.

In male and female mice, degenerative changes of the respiratory epithelium of the nasal cavity were reported at doses >=100 mg/kg body weight per day and degenerative changes of the respiratory nasopharynx

at doses >=300 mg/kg body weight per day.

Other findings included variations in serum enzyme levels (increase in alkaline phosphatase, aspartate transaminase, and alanine transaminase) and an increased urea nitrogen level in the low-dose males and females, which became more pronounced with increasing doses. In female mice receiving >=300 mg biphenyl/ kg body weight per day and in the high-dose males, degenerative changes in the kidney (increased mineralization of the inner stripe of the outer medulla, increase in desquamation of the epithelium of the renal pelvis) were also observed. High-dose animals also showed reduced body weight gain and food consumption.

** MALE MICE **

	DOSE			
End-point	0 mg/kg	667 mg/kg	2000 mg/kg	6000 mg/kg
survival rate	35/50	41/50	41/50	39/50
hepatocellular carcinoma	8/50	8/49	5/50	4/50
hepatocellular adenoma	8/50	6/49	7/50	3/50
basophilic cell foci	0/50	6/49	1/50	2/50

Historical control data:
carcinoma: 171/700 with a range of 1/50 - 19/50
adenoma: 119/700 with a range of 2/50 - 15/50

** FEMALE MICE **

	DOSE			
End-point	0 mg/kg	667 mg/kg	2000 mg/kg	6000 mg/kg
survival rate	31/50	22/50	25/50	32/49
hepatocellular carcinoma	1/50	5/50 (p=0.12)	7/50 (p=0.043)	5/49 (p=0.12)
hepatocellular adenoma	2/50	3/50 (p=0.49)	12/50 (p=0.016)	10/49 (p=0.025)
basophilic cell foci	1/50	1/50	12/50	6/49

Historical control data:
carcinoma: 15/699 with a range of 0/50 - 2/50
adenoma: 33/699 with a range of 1/50 - 5/50

Test substance : Biphenyl, CASNO 92-52-4
Conclusion :

The results did not show a carcinogenic effect in mice exposed to a "maximum tolerated dose" of biphenyl in feed.

Degenerative changes of nasal cavity respiratory epithelium were reported at doses >=100 mg/kg body weight per day and a variation in serum enzymes suggestive of liver and kidney effects were the most sensitive end point. A NOAEL was not identified for these effects.

Degenerative changes of the respiratory nasopharynx at doses >=300 mg/kg body weight per day are likely part of a continuum of effects possibly mediated by vapor exposure to biphenyl while eating.

Degenerative kidney changes were observed in female mice receiving >=300 mg biphenyl/kg body weight per day and in the high-dose males.

Reliability : (1) valid without restriction

Modern guideline study under GLP's with sufficient documentation.

14.12.2003 (20)

Type : Sub-chronic
Species : mouse
Sex : male/female
Strain : CD-1
Route of admin. : inhalation
Exposure period : 13 weeks
Frequency of treatm. : 7 hours/day 5 day/wk
Post exposure period : 30 days
Doses : 25 and 50 ppm
Control group : yes, concurrent vehicle
LOAEL : = 25 ppm
Method :
Year :
GLP : no
Test substance :

Method :

A 13-week vapor inhalation study using groups of 50 CD-1 mice of each sex exposed to 25 or 50 ppm (160 or 320 mg/m3; analytical concentrations) biphenyl for 7 hours/day, 5 days/week was conducted. Mice were obtained as weanlings (5-25 grams) and received food and water ad libitum except during the 7-hour exposures.

Exposures were conducted in 0.5 cubic meter stainless steel "Rochester" type chambers with glass windows on all four sides for viewing. During the exposures, 10 mice of one sex were housed in a cage with a divider such that 5 mice were together on one side of the cage. Cages were placed on a raised wire mesh floor in the exposure chamber.

Biphenyl vapor was generated by heating biphenyl, contained in a three-necked flask, in an oil-bath while directing air in one of the necks and out another through a heated connector tube to the chamber. Airflow was maintained at 2 L per minute. The concentration of test material in the chambers was determined twice daily by drawing a known volume of vapor through two impingers in tandem containing cyclohexane. The solutions were analyzed for biphenyl by uv against a standard curve.

Mice were observed during the exposure for adverse clinical signs and were weighed weekly. Near the end of the study, each group of surviving mice was places in a metabolism cage for a 12-hour urine collection. Blood for hematology was collected at sacrifice from the dorsal vein after opening the pleural cavity.

Ten mice of each sex from each group were held for a 30-day recovery period prior to sacrifice.

Some difficulties occurred maintaining the biphenyl level during the first weeks of exposure but these issues were solved and exposure control was tighter during the remainder of the study. The average concentration of biphenyl in the chambers was 25 ± 7 ppm (26.5 ± 1 ppm during the last 72 days) and 50 ± 16 ppm (51.4 ± 9.6 ppm during the last 55 days).

Due to a technical problem several mice in the 25-ppm exposure group were inadvertently killed at week 12 of the study when they were overheated in a holding room. These mice were replaced with unexposed weanling mice and the entire group was exposed until the replacement mice had received 65 exposures.

At study termination all surviving animals were submitted to a gross examination and tissues of the following organs were collected, prepared and microscopically examined: trachea, lungs, livers, kidney and spleens.

Result :

Exposed mice weight gain was comparable to controls throughout the study. An explicit table giving mortality was not included in the report. Due to the inclusion of the replacement mice and the poor legibility of the tables the mortality could not be determined from the tables of individual animal weights. Mortality per group could not be reliably ascertained from the pathology report, which noted that only 71 high-dose animals and 98 low-dose animals were available for examination at study termination. This suggests some compound-related mortality in the high-dose group but the extent cannot be verified.

Clinical chemistry parameters were SGOT, SGPT, alkaline phosphatase, bilirubin, uric acid and BUN. Statistical analysis was not presented in the report and, due to poor legibility, post-hoc analysis was impossible. Examination of the results suggests that SGOT and SGPT were elevated in high-dose animals of each sex sacrificed at the end of 13-weeks exposure while all other parameters were unremarkable. Not enough blood was obtained from the 25-ppm males to allow clinical chemistry. Clinical chemical determinations were conducted after the 30-day recovery period but only on two animals per group. In these 4 (2 of each sex) 50-ppm animals, the SGPT and SGOT levels were similar to controls.

Except for a possible increase in white blood cells in 25-ppm group females at the end of the exposure period, hematology values were unremarkable. Blood from all 25-ppm males was hemolized and no data were recorded for this group. Hematology was also conducted on two mice from each 30-day recovery group and the results were unremarkable; however, with the limited sample, no conclusions can be drawn.

At gross examination, a finding of congested lungs or lungs hemorrhagic were reported in the majority of high-dose animals, about half of the low-

dose animals and about 10 percent of control animals. With the exception of sporadic findings of "small spleen", no gross changes were recorded except for the lungs.

Microscopic examination resulted in a diagnosis of hyperplasia with inflammation of the trachea for 70/71 high-dose animals, 80/89 low-dose animals and 0/80 controls. After 30 days of recovery, the incidence of hyperplasia with inflammation of the trachea was 5/19 at the high dose, 2/15 at the low dose and 3/20 in controls. Congestion of the lungs, liver and kidneys observed in several animals at microscopic examination was attributed by the pathologist to an effect of the anesthetic used for sacrifice. Congestion and edema of the lungs was found with incidence similar to hyperplasia of the trachea; however, based on the pathologists remark about the congestion being related to anesthetic administration at sacrifice, it cannot be determined if this was compound related.

Test substance :

Biphenyl, CASNO 92-52-4, purity 99%

Conclusion :

Inhalation of biphenyl vapor for 13-weeks results in marked respiratory tract inflammation and hyperplasia of the trachea in mice of each sex at 50 ppm with 25 ppm being a LOAEL. The effects appear to be partially reversible after a 30-day recovery period. A NOAEL was not identified.

Reliability : (4) not assignable

Due to limited scope and technical difficulties the overall study is assigned a reliability of 4; however, the histopathological examination and reporting of findings to the trachea is considered to have a higher reliability.

14.12.2003 (11)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Bacterial reverse mutation assay
System of testing	:	Salmonella typhimurium
Test concentration	:	0 to 100 micrograms per plate
Cycotoxic concentr.	:	100 micrograms/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: NTP
Year	:	
GLP	:	no data
Test substance	:	

Method :

As each strain of Salmonella typhimurium is genetically different, using several strains in a test increases the opportunity of detecting a mutagenic chemical. All strains of Salmonella typhimurium used for mutagenicity testing carry a defective (mutant) gene that prevents them from synthesizing the essential amino acid histidine. Mutations leading to the ability to sysntesize histidine are called "back" or "reverse" mutations and the process is referred to as "reversion."

Some test protocols utilize extracts of Aroclor rat or hamster liver enzymes (S9) to promote metabolic conversion of the test chemical. This is necessary since the Salmonella bacterium does not have the mamillian metabolic capabilities.

In the Salmonella assay, a test tube containing a suspension of one strain of Salmonella typhimurium plus S9 mix or plain buffer without S9, is incubated for 20 minutes at 37º C with the test chemical. Control cultures, with all the same ingredients except the test chemical, are also identically incubated. In addition, positive controls with a known potent mutagen, are prepared. After 20 minutes, agar is added to the cultures and the contents of the tubes are thoroughly mixed and poured onto the surface of petri dishes containing standard bacterial culture medium. The plates are incubated, and bacterial colonies that do not require an excess of supplemental histidine appear and grow. These colonies are comprised of Salmonella that have undergone reverse mutation to restore function of the histidine-manufacturing gene. The number of colonies is counted after 2 days.

Several doses (at least 5) of each test chemical and multiple strains of Salmonella typhimurium are used in each experiment. In addition, cultures are set up with and without added S9 liver enzymes at 10% concentration in these studies.

The pattern and the strength of the mutant response are taken into account in determining the mutagenicity of a chemical. All observed responses are verified in repeat tests. If no increase in mutant colonies is seen after testing several strains under several different culture conditions, the test chemical is considered to be nonmutagenic in the Salmonella test.

Reference
Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000 Nov 20;455(1-2):29-60.

Remark :

This result is also supported by the following reports of negative Ames tests on Biphenyl:

Bos RP, Theuws JLG, Jongeneelen FJ, Henderson PT (1988) Mutagenicity of bi-, tri- and tetra-cyclic aromatic hydrocarbons in the "taped-plate assay" and in the conventional Salmonella mutagenicity assay. Mutation research, 204:203-206.

Brams A, Buchet JP, Crutzen-Fayt MC, de Meester C, Lauwerys R, Leonard A (1987) A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure). Toxicology letters, 38:123-133.

Fujita H, Kojima A, Sasaki M, Hiraga K (1985) Mutagenicity test of antioxidants and fungicides with Salmonella typhimurium TA97a, TA102. Kenkyu Nenpo-Tokyo-toritsu Eisei Kenkyusho, 36:413-417.

Glatt H, Anklam E, Robertson LW (1992) Biphenyl and fluorinated derivatives: liver enzyme-mediated mutagenicity detected in Salmonella typhimurium and Chinese hamster V79 cells. Mutation research, 281:151-156

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983) Salmonella mutagenicity test results for 250 chemicals. Environmental mutagenesis, 5 (Suppl. 1):3-142.

Ishidate M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M, Matsuoka A (1984) Primary mutagenicity screening of food additives currently used in Japan. Food and chemical toxicology, 22:623-636.

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Bronzetti G, Esposito A, Pagano G, Quinto I (1981) A comparative study on the toxicity and mutagenicity of biphenyl (BP) and diphenyl ether (DPE) in sea urchin, S. typhimurium and S. cerevisiae. Mutation research, 85:233.

Cline JC, McMahon RE (1977) Detection of chemical mutagens. Use of concentration gradient plates in a high capacity screen. Research communications in chemical pathology and pharmacology, 16:523-533.

Pagano G, Esposito A, Giordano GG, Vamvakinos E, Quinto I, Bronzetti G, Bauer C, Corsi C, Nieri R, Ciajolo A (1983) Genotoxicity and teratogenicity of diphenyl and diphenyl ether: a study of sea urchins, yeast, and Salmonella typhimurium. Teratogenesis, carcinogenesis, and mutagenesis, 3:377-393.

Pagano G, Cipollaro M, Corsale G, Della Morte R, Esposito A, Giordano GG, Micallo G, Quinto I, Staiano N (1988) Comparative toxicity of diphenyl, diphenyl ester, and some of their hydroxy derivatives. Médecine Biologie Environnement, 16:291-297.

Result :

Data found on NTP public database at http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm

Study ID 512660
Solvent DMSO
Preincubation

Strain: TA100

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	173	24.3	116	3.8	146	2.8	157	7.8	156	10.9	155	7.5
1	173	14.8	110	6.9	169	13	182	0.7	165	6.5	164	6.6
3.3	176	15.3	103	3.4	158	7.9	160	7.8	153	3.6	189	18.3
10	146	23.2	101	10	151	3.3	164	2.8	164	3.4	118	45
33	135	51.7	85	6.2	152	4.9	165	2.2	151	12.5	175	13.6
100	90	42.5	67	9	154	9.9	163	15.1	160	6.7	161	12.7
P Con	447	32.4	375	19.9	297	5.7	357	14.9	641	81	587	49.8

Strain: TA1535

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	12	1.2	4	0.9	10	0.3	9	0.7	13	0.6	6	0.9
1	11	1.2	7	1.8	6	1	6	0.3	11	0.3	4	0.9
3.3	9	1.5	6	1.5	6	1.3	6	1	9	2	4	0
10	5	1.5	4	1.9	8	1	6	0.9	7	1.5	4	0.7
33	10	2.3	5	0.9	6	2.8	6	0.7	7	1.5	6	1.7
100	11	1.9	5	1.2	9	2.1	7	0.6	9	0.3	9	2.4
P Con	269	9.1	333	25.9	29	4.8	18	0.6	42	7.2	29	2.7

Strain: TA1537

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	7	1	6	0.9	9	0.6	9	1.2	9	1.2	9	0.6
1	5	1.5	6	1.5	6	1.5	12	3	7	1.5	8	0.7
3.3	5	0.9	5	0.9	5	0.9	11	3.2	8	1.2	8	0.6
10	7	0.6	6	1.2	8	1.2	8	0.3	5	0.6	8	1.5
33	3	0.6	6	1.7	7	1.5	7	0.3	8	2.3	8	0.3
100	4	1.5	6	1.5	7	1.2	7	0.3	6	1.2	6	0.6
P Con	297	31.7	122	5.8	21	3.5	36	11.6	75	16.5	64	3.9

Strain: TA98

Dose	No MA (neg.)		No MA (neg.)		RLI (neg.)		RLI (neg.)		HLI (neg.)		HLI (neg.)	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	15	1.5	14	0.6	19	1.2	19	1.2	19	3.7	19	6.3
1	15	3	15	1.5	19	3.1	23	2.3	19	1.9	28	4.6
3.3	13	1.5	11	1.3	22	5.4	20	1.2	20	1.7	31	5.9
10	11	3.5	12	1.2	18	2.1	15	1.5	18	1.7	29	6.4
33	8	1.3	13	3.8	20	1.7	21	1.8	16	2.7	26	5
100	7	0.6	13	3.5	22	0.9	17	2.6	19	3.5	23	2.2
P Con	283	14.2	266	4.5	189	16	333	22.4	610	93.7	718	101

Study ID 773612
Solvent DMSO
Preincubation

Strain: TA100

Dose	No MA		No MA		RLI		RLI		HLI		HLI	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	102	3.2	73	4.4	91	3.2	86	2.5	88	4.1	68	4.1
1	109	6.9	78	3.7	97	5	84	4.6	82	3.8	73	5.5
3	92	7.4	76	2.9	93	4.7	79	1	85	0.9	63	3.5
10	103	7.8	81	5	94	9.9	73	0.3	84	10.5	62	3.2
33	76	2.9	59	3.5	87	8.6	74	7	89	0.7	74	3.9
100	87s	0.9	58	6.4	93	5.3	60	0.3	86	4	61	4.4
P con	1955	92	1864	48.3	493	57	1432	62.5	3349	123	2649	267

Strain: TA1535

Dose	No MA		No MA		RLI		RLI		HLI		HLI	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	9	3	18	1.2	7	1.2	10	0.9	7	2.2	12	0.9
1	12	0	18	2.1	12	3.2	9	2	11	0.3	11	0.3
3	10	1.2	18	2	8	1.5	10	1.2	13	1.5	5	0.9
10	8	2.6	23	4	8	0.3	9	1.2	9	2	11	2
33	5	1.9	17	2.6	11	1	8	2.2	10	2.3	8	2
100	179	0	14s	0.9	5s	2	9	1.8	6s	1.3	8s	1
P con	857	19	887	142	55	4.7	81	3.3	295	11.1	123	8.8

Strain: TA1537

Dose	No MA		No MA		RLI		RLI		HLI		HLI	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	6	1.2	3	0.9	5	0.9	9	3.2	6	0.3	7	1.2
1	7	1.2	5	0.9	7	0.7	7	0.3	6	1.5	7	1.9
3	5	0.9	9	1.5	9	1.7	5	1.5	7	0.9	4	0.9
10	7	0.7	7	2.5	7	2.8	6	1.5	5	0.3	6	1.7
33	4	1.5	3	1.7	7	1.5	7	2.8	5	0.3	7	1.5
100	3s	0.7	3s	1	6	2	5	1.2	6	0	4	1.5
P con	503	93	192	65.3	64	16	55	2.6	285	9	255	5.8

Strain: TA98

Dose	No MA		No MA		RLI		RLI		HLI		HLI	
ug/P	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem	Mean	sem
0	16	1.8	27	5.2	16	0.6	21	3.9	25	5	25	2.6
1	15	1	22	3.3	19	1.9	22	2.4	21	0.3	21	2.7
3	17	0.6	22	3.8	19	2.5	24	1.2	24	1.2	18	2.7
10	19	3.4	20	2.6	16	1.5	22	4	19	1.3	19	2.1
33	12	2.6	11	1.2	19	1	20	2.3	19	4.7	23	2.5
100	10s	0.9	12	1.2	20	1.2	18	1.2	20	2.3	19	2.1
P con	1207	34	1564	29.2	384	48	1322	35.8	2525	251	2748	127

S = Slight Toxicity
MA = Metabolic Activation
RLI = Rat Liver, Induced
HLI = Hamster Liver, Induced

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Material was non-mutagenic in the presence or absence of a standard liver metabolic activating system

Reliability : (1) valid without restriction

High quality study with multiple-species activating system and independent confirmation.

Flag : Critical study for SIDS endpoint

14.12.2003 (13)

Type : Cytogenetic assay
System of testing : Syrian Hamster cell line: DON
Test concentration : 0.1, 0.2, 0.5 or 1.0 mM
Cycotoxic concentr. : 1.0 mM showed mitotic inhibition
Metabolic activation : without
Result : negative
Method :
Year :
GLP : no
Test substance :

Method :

The purpose of this study was to compare chromosome aberrations and sister chromatid exchange frequency for several chemicals under identical culture conditions. In this study a pseudodiploid Chinese hamster cell line (Don) was exposed using three to five concentrations of the test materials. In the case of Biphenyl, concentrations of 0.1, 0.2, 0.5 or 1.0 mM test material were incubated with cells in Eagle's MEM with 10% FCS and 1 microgram per ml BudR for 26 hours (2 rounds of cell division) at 37° C in compete darkness. Colchicine (0.25 mcgm/ml) was added for the last two hours of incubation. Cell were collected using a rubber policeman and air-dries slides were prepared following hypotonic treatment for 20 minutes and fixation in ice-cold methanol:acetic acid (3:1). Slides of chromosome aberration examination were prepared by conventional Giemsa staining. Separate slides received special stains for determining SCEs.

Slides were cored by examining 100 metaphases for each concentration and the frequency of aberrations, excluding gaps, was estimated by the number of breaks per cell. A ring, a dicentric and a chromatid exchange were each scored as two breaks, a tricentric as four breaks, and an

acentric or isochromatid break were scored as one break.

SCE's were scored by a different investigator and 20-50 intact metaphases per concentration in which all metaphases had a "harlequinized" appearance without gross chromosome aberraion

The criterion for a positive result was set at a dosage-related increase in aberrations of at least twice that of controls. Positive substances were also run as part of the study.

Remark :

ASSAY	ENDPOINT	CONCENTRAT RANGE	RESULT A	RESULT B	REF
Mouse lymphoma assay	Gene mutation	0-61 µg/ml	-	(+)	[i]
Chinese hamster cells (CHL)	Chrom aberr	0-125 µg/ml	-	0	[ii][iii][iv]
Chinese hamster cells(CHL)	Chrom aberr	0-20 µg/ml	-	+	[v]
Chinese hamster cells (Don)	Chrom aberr	15.4-154 µg/ml	-	0	[vi]
Rat hepatocytes [vii][viii][ix]	UDS	.002-154 µg/ml	0	-	
Chinese hamster cells(CHL)	SCE	no data	-	0	[iii]
Chinese hamster cells (Don)	SCE SCE	15.4-154 µg/ml	-	0	[x]
L5178Y cells DNA unwinding	DNA damage	0-231 µg/ml	-	+	[xi]
Human lung fibroblasts WI-38 cells)	UDS	no data	-	-	[xii]
Human fibroblasts nick translation assay	DNA damage	15.4 µg/ml	-	0	[xiii]

RESULTS
A = No metabolic activation; B = in presence of S9 system
0 = no data
+ = positive,
- = negative
(+) = equivocal or weak positive

REFERENCES SUPPORTING CA RESULT

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Mitchell AD, Jorgenson TA, Jones DCL, Valencia R, Garrett NE (1982) Study of pesticide genotoxicity. Basic life sciences, 21:275-326.

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Result :

The high concentration (1.0 mM) produced some toxicity as evidenced by an inhibition of mitotic activity.

Results of the scoring for "breaks" and "exchanges" are:

Conc (mM)	breaks/cell	SCE/cell
0.0	0.06	8.17
0.1	0.10	10.37
0.3	0.12	9.06
0.5	0.03	10.33
1.0	0.08	13.12
pos cont*	>7.77	18.44

Positive control was N-n-butyl-N-nitrosourethane at 1 mM for chromosome aberrations and at 0.1 mM for SCE.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Biphenyl did not produce an increase in chromosome aberrations or SCEs under these conditions, negative and positive controls gave the expected results.

Reliability : (2) valid with restrictions

Published reports are assigned a reliability of 2. Despite differences from the current OECD 473 guidance, the information is considered reliable, as results of a large range of compounds were available providing validation of the methodology. Differences form OECD 473 were that there was no metabolic activation system used, cytotoxicity was not determined and it may have been possible to use a higher concentration (10 mM is the highest concentration recommended by the OECD 473 guideline) and the number of metaphases examined was half that recommended by the current guideline.

Flag : Critical study for SIDS endpoint

14.12.2003 (2)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type	:	other: Three generation study
Species	:	rat
Sex	:	
Strain	:	Long-Evans
Route of admin.	:	oral feed
Exposure period	:	lifetime
Frequency of treatm.	:	Cont
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	3
Doses	:	100, 1000 or 10000 ppm
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 1000 ppm
NOAEL F1 offspring	:	= 1000 ppm
NOAEL F2 offspring	:	= 1000 ppm
Result	:	Not Specific Reproductive Toxin

Method :

In this multigeneration test, weanling Long Evans rats of each sex were raised on a basal control diet until approximately four months of age at which time they were then divided into groups of three males and nine females each and fed the following diets:

Group 1: Control basal diet
Group 2: Basal diet containing 0.01% Biphenyl (100 ppm)
Group 3: Basal diet containing 0.1% Biphenyl (1000 ppm)
Group 4: Basal diet containing 01.0% Biphenyl (10,000 ppm)

For breeding, three females and-one male-were placed together in wire bottom cage. They were housed in air-conditioned animal quarters maintained at 73-77F and 45-50% relative humidity with diets and water available ad lib. Breeding females not observed to be pregnant after four weeks were placed with another male of the same group. If no pregnancy resulted after a total of nine weeks, the female was recorded as sterile. Females observed to be pregnant were placed in individual cages with nesting material. Litter size was recorded at birth.

At two days of age, the young were weighed and reduced to seven per litter. Pups were weaned at three weeks of age and weighed weekly from the third through the sixth week of life.

Young (Generation 2) from the first generation rats were continued after weaning on-the same diets that their parents had received. At ten weeks of age, nine females and three males of the second generation were mated. In turn, their offspring (Generation 3) were treated as above and in-turn they were mated to produce the fourth generation. Fourth generation rats were sacrificed at three weeks of age and twelve animals from each diet group autopsied for gross pathology

Remark :

Although feed consumption data and breeding rat weight data are not available, the hypothesis that the high-dose effects are related to reduced

food consumption due to palatability is supported by the 1960 Ambrose feeding study where diets containing 5000 and 10000 ppm Biphenyl were shown to result in reduced food consumptions and reduced body weight gain.

Result :

Rats that were maintained on diets containing 100 or 1000 ppm Biphenyl had a reproduction record entirely consistent with the control rats in respect to fertility, lactation, size of litter, growth and mortality of the pups. Reproductive performance through three generations of exposure showed no cumulative effect of treatment and all rats of the forth generation were unremarkable at sacrifice and necropsy.

Data for dams are as follows:

DIET	Gen	Dams Bred	Litters Cast	Days mating to littering
Control	1	9	8	24
	2	9	8	28
	3	9	8	26
100 ppm	1	9	8	32
	2	9	9	31
	3	9	9	31
1000 ppm	1	9	9	29
	2	9	9	28
	3	9	9	27
10000 ppm	1	9	6	33
	2	9	7	33
	3	9	8	31

Data for pups:

		# Pups litter	Mean weight pups (g)					Mean Litter Size	
DIET	Gen	mean	2d	3w	4w	5w	6w	3w	6w
Control	2	8.4	8.8	48	74	90	104	6.1	4.7
	3	7.3	7.7	45	70	95	122	6.6	5.0
	4	10.2	8.0	50				7.0	
100 ppm	2	8.6	7.0	50	59	88	110	6.4	5.6
	3	9.3	8.6	45	66	97	125	6.2	6.2
	4	11.3	8.1	44				7.0	
1000 ppm	2	7.0	8.3	47		81	87	5.7	5.4
	3	8.4	7.7	44	64	95	123	6.4	5.7
	4	8.3	8.6	46				5.6	
10000 ppm	2	5.7	8.6	35		64		5.0	4.2
	3	5.4	7.1	36	49	69	91	4.7	4.4
	4	7.4	7.0	32				6.5	

Administration of the 10000 ppm Biphenyl diet proved to have adverse effects on reproductive parameters. Fertility of the females was decreased from an average of 8.3 litters for the controls to 7.0 liters (mena. The mean litter size was significantly (statistically) smaller with an average of 8.6 pups per litter for controls and 6. 2 pups/litter in the 10,000 ppm group. Body weights of pups fed diets with 10000 ppm Biphenyl were statistically less than the control rats at both three and six weeks of age. All rats appeared normal at

Necropsy and there was no evidence of cumulative toxicity over the three generations studied.

It was suggested that the adverse effects on fertility may have been caused by unpalatability of the diet resulting in lower food consumption rather than by any effect of the test substance on physiological function.

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Marginally reduced fertility occurred at feeding levels that were toxic to the young adult animals as manifest by reduction in weight gains. Feed levels that were not associated with parental toxicity did not have any effect on reproductive parameters over four generations of exposure. Biphenyl is not a specific reproductive toxin to the rat.

Reliability : (2) valid with restrictions

Although this study lacks some details and it was conducted by a scientifically defensible method and is considered to have good reliability. Another strength of the study is that there was a clear maternally and paternally toxic dose tested that produced only small effects on reproductive parameters.

Flag : Critical study for SIDS endpoint

05.11.2003 (24)

Type : Fertility
Species : rat
Sex : male/female
Strain :
Route of admin. : oral feed
Exposure period : 11 or 60 days before mating through weaning
Frequency of treatm. : Cont
Premating exposure period
Male : 11 or 60 days
Female : 11 or 60 days
Duration of test :
No. of generation studies : 1
Doses : 1000 or 5000 ppm
Control group : yes, concurrent vehicle
NOAEL parental : = 1000 ppm
NOAEL F1 offspring : = 5000 ppm

Method : Groups of 15 weanling rats of each sex were placed on diets containing seven levels of biphenyl for a period of 750 days. In the main study, animals were housed 5 to a cage and had free access to food and water at all times. During the period of growth, rats were weighed and food consumption was determined weekly. Following the period of active growth, the rats were weighed at 50-day intervals for the duration of the study. Animals were examined at the time of weighing for gross evidence of tumors. At sacrifice, animals were necropsied, weights of liver, kidneys, heart, and testes were determined. Hematoxylin-eosin stained sections of heart, lung, liver, kidney, adrenal, spleen, pancreas, stomach, intestine, bladder, thyroid, brain, pituitary, and gonads were prepared and bone marrow smears of representative animals were prepared.

Dosed feed levels for the study were 0, 10, 50, 100, 500, 1000, 5000 or 10000 ppm (0.001 to 1%).

Studies on possible reproductive effects and survival of young were also conducted as follows. Ten weanling female and five male rats were placed on control diet for 60 days, and subsequently mated, one male to two females. An identical experiment included Biphenyl at a dietary level of 0.1%. Nine female and 3 male rats were fed a dietary level of 0.5% Biphenyl in a subsequent study. All rats continued exposure until the pups of all litters were weaned.

In a second series of reproductive experiments, 90-day old rats were exposed for 11 days before mating and continuously until weaning of pups. Using this dosing schedule, 8 female and 4 male rats were placed on the control diet, 8 females and 4 males received 0.1%, and 9 females and 3 males received 0.5% dietary levels of Biphenyl.

Result :

Two studies of potential reproductive effects and survival of young were conducted. In the first, male and female animals were treated for 60 days pre-mating with diets containing 0, 5000, or 10000 ppm Biphenyl. Dams continued exposure until weaning of pups. The group sizes are shown in the results table

STUDY 1: 60-Day Pre-mating Treatment.

Conc	Females Mated	Females delivering	Total pups	Range of litter size	pups litter
0	10	9	59	3-9	6.5
5000	10	10	67	2-10	6.7
10000	9	8	53	3-9	6.6

In the second study, 90-day old rats of each sex were exposed for 11 days before mating and continuously until weaning of pups. The group sizes are shown in the results table

STUDY 2: 11-Day Pre-mating Treatment.

Conc	Females Mated	Females delivering	Total pups	Range of litter size	pups litter
0	8	8	64	5-13	8.0
5000	8	6	63	3-10	10.5
10000	9	8	48	3-9	6.0

Statistical analysis of reproductive data was not presented. It was concluded that "Dietary levels of 0.1 and 0.5% Biphenyl had no significant effect on reproduction."

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

No effect on reproductive ability or pup survival was found.

Reliability : (2) valid with restrictions

Study limited in scope, information about fertility and pup survival valuable but not definitive due to lack of modern end-point parameters.

05.11.2003 (5)

Type	:	Fertility
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344/DuCrj
Route of admin.	:	oral feed
Exposure period	:	2 Years
Frequency of treatm.	:	Continuous
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	38, 113, or 338 mg/kg-day
Control group	:	yes, concurrent vehicle

Method :

Two-year carcinogenicity studies were conducted using rats and mice of each sex by the Japan Bioassay Research Center. In these studies rats were fed biphenyl in the diet at a levels such that the average dose over the two-year bioassay was 38, 113, or 338 mg/kg-day for rats of each sex. Mice, likewise received biphenyl containing feed for a period of two-years at feed concentrations such that the dose levels were 100, 300 or 900 mg/kg-day. The initial group size for this study was 50 animals per sex for each dose level. The survival rate was high with approximately 80 % of male mice, 60% of female mice, 75% of male rats and 80% of female rats surviving.

The dosage levels were selected based on a subchronic evaluation in rats and mice and were set to represent the maximum-tolerated dose (MTD) to provide a robust test for carcinogenic potential of biphenyl. Information concerning the long-term effects of biphenyl on a variety of other organ systems is also obtained from the two-year bioassays because animals receive a "complete" necropsy, and an extensive and generally standardized list of tissues are examined by gross and microscopic means. The report containing the organ list for microscopic examination was not available for review but is can safely be assumed that the reproductive organs were given a through examination. This is confirmed in the WHO IPCS CICAD document in which the results of the two-year bioassay are presented in considerable detail and it is noted specifically that: "Histopathological changes within the male and female reproductive systems were not observed in rats or mice administered biphenyl at 400-4500 mg/kg in the diet for 2 years". In a modern guideline carcinogenicity study such as was conducted on biphenyl, the following reproductive organs are routinely microscopically examined in at least high-dose and control animals.

- epididymides
- mammary gland
- ovaries
- pituitary gland
- preputial glands
- prostate
- seminal vesicle
- testes
- thyroid
- uterus

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

Administration of dietary concentrations of biphenyl to F344/DuCrj rats of each sex sufficient to cause frank organ toxicity in the bladder, kidneys and other organ systems did not result in any observable adverse effect on reproductive organs

Reliability : (2) valid with restrictions

Although satisfactory guideline GLP study, downgraded to 2 as information is obtained from secondary literature.

14.12.2003 (12)

Type : other: Chronic
Species : mouse
Sex : male/female
Strain : other: Cjr:BDF1
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatm. : Continuous
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 100, 300 or 900 mg/kg-day
Control group :

Method :

Two-year carcinogenicity studies were conducted using rats and mice of each sex by the Japan Bioassay Research Center. In these studies rats were fed biphenyl in the diet at a levels such that the average dose over the two-year bioassay was 38, 113, or 338 mg/kg-day for rats of each sex. Mice, likewise received biphenyl containing feed for a period of two-years at feed concentrations such that the dose levels were 100, 300 or 900 mg/kg-day. The initial group size for this study was 50 animals per sex for each dose level. The survival rate was high with approximately 80 % of male mice, 60% of female mice, 75% of male rats and 80% of female rats surviving. The dosage levels were selected based on a subchronic evaluation in rats and mice and were set to represent the maximum-tolerated dose (MTD) to provide a robust test for carcinogenic potential of biphenyl. Information concerning the long-term effects of biphenyl on a variety of other organ systems is also obtained from the two-year bioassays because animals receive a "complete" necropsy, and an extensive and generally standardized list of tissues are examined by gross and microscopic means. In the case of biphenyl report the report containing the organ list for microscopic examination was not available for review but is can safely be assumed that the reproductive organs were given a through examination. This is confirmed in the WHO IPCS CICAD document in which the results of the two-year bioassay are presented in considerable detail and it is noted specifically that: "Histopathological changes within the male and female reproductive systems were not observed in rats or mice administered biphenyl at 400-4500 mg/kg in the diet for 2 years". In a modern guideline carcinogenicity study such as was conducted on biphenyl, the following reproductive organs are routinely microscopically examined in at least high-dose and control animals.

- epididymides
- mammary gland
- ovaries
- pituitary gland
- preputial glands
- prostate
- seminal vesicle
- testes
- thyroid
- uterus

Test substance :

Biphenyl CASNO 92-52-4, purity > 99.1%

Conclusion :

Administration of dietary concentrations of biphenyl to Cjr:BDF1 mice of each sex sufficient to cause a reduction in body weight gain did not result in any observable adverse effect on reproductive organs

Reliability : (2) valid with restrictions

Although satisfactory guideline GLP study, downgraded to 2 as information is obtained from secondary literature.

13.12.2003 (12)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	day 6-15 of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	125, 250, 500, 1000 mg/kg bw
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 500 mg/kg bw
NOAEL teratogen.	:	= 500 mg/kg bw
Result	:	Not specific developmental toxin in this study
Method	:	
Year	:	
GLP	:	no data
Test substance	:	

Method : Female Wistar rats, 175-200 g body weight, were paired overnight with proven males. The morning a positive vaginal smear was observed was counted as Day 1 of gestation. Eighteen to 20 mated females were assigned to each dosage group and a control group was included.

Females were weighed on the 1st, 6th through 15th, and 22nd days of pregnancy. At sacrifice on day 22 of gestation, the carcass was before and after the uterine contents were removed, the number of corpora lutea were determined, and a necropsy performed. The fetuses were weighed and examined for viability and external malformations. Early resorption or implantation sites and fetuses dying at a late stage in their development were recorded as dead fetuses. Two-thirds of the live fetuses from each litter were studied for skeletal development following alizarin red staining). The remaining fetuses were fixed in Bouin's fluid, sectioned at 1-mm intervals with a razor blade, and examined for visceral anomalies.

Test material was administered on days 6 through 15 of gestation by gavage using corn oil as vehicle. Dose levels were selected on the basis of a preliminary experiment in which dosed of 2000 mg/kg resulted in the death of all dams 2-3 days after initiation of dosing. The dosing volume 10 ml/kg body weight and the doses employed were 0, 125, 250, 500 or 1000 mg/kg.

Statistical methods. In assessing effects of treatment on maternal body weight, mean and SE were calculated for each experimental group and t values were obtained for test group versus control group differences in means. The litter was treated as the basic observational unit for analysis of fetal parameters, and the proportion of a litter having a particular effect was calculated. The mean and its SE of the proportion in the different test groups, were derived. The t test was used for comparison of test and control values and differences were considered to be significant at $p < 0.05$.

Result :

Maternal Effects: In the animals receiving the highest dose, 1000 mg/ kg, it was found that resorption occurred in one litter, five animals were found not to be pregnant (which may have been due to interference with implantation), and mortality occurred in an additional five females. Each death occurred during the dosing period and was preceded by a sharp reduction in body weight and diarrhea. The remaining doses of biphenyl, 125, 250, and 500 mg/kg, elicited no signs of toxicity. Maternal body weights were only presented graphically in the publication. Examination of the graph indicates reduction in body weight gain only at the 1000 mg/kg level.

Fetal and Related Effects: at the 1000 mg; kg dose Biphenyl was lethal for five dams; however, in those that survived, it did not affect the incidence of corpora lutea, live fetuses, or dead fetuses plus resorption sites, nor did it affect fetal weight. Although fetal weight was reduced, and the incidence of dead fetuses plus resorptions increased, these values were not significantly different from control animals. In the 1000 and 500 mg/kg groups, there was a slight increase in the number of fetuses with missing and unossified sternebrae or with delayed calvarial ossification but these increases were not statistically significant.

	DOSE LEVEL				
	0	125	250	500	1000
EFFECT					
Number of rats with live Fetuses at term/number mated	6/18	20/20	18/19	18/20	9/20
Number of corpora lutea per pregnancy	12.6±0.4	12.9±0.4	13.7±0.5	13.3±0.4	12.5±0.7
Number of live fetuses per pregnancy	11.3±0.7	11.8±0.6	11.9±0.6	11.2±0.5	10.7±1.3
Dead and resorbed fetuses	4.8	3.3	6.1	7.8	13.7
Fetal weight (g mean ±SE)	5.1±0.1	5.3±0.1	5.2±0.1	5.2±0.1	4.5±0.3
Number of anomalous fetuses	17/176	22/236	22/213	35/199	25/107
Number of anomalous litters	8/16	11//20	13/18	15/18	37781
ANOMALIES (#fetuses affected)					
Wavy ribs, uni- and bilater	3	7	9	8	5
Extra ribs, uni and bilater	9	12	9	15	6
13th rib, small sized	1	1	2	1	0
Sternebrae, missing or unos	4	3	4	16	17
Calvarium, delayed ossifica	0	2	0	0	8
Miscellaneous	1	1	1	0	0

Test substance :

Biphenyl, CASNO 92-52-4

Conclusion :

The maternal and fetal NOEL is 500 mg/kg. In spite of severe maternal toxicity at 1000 mg/kg, there was only minor fetotoxicty produced at this level. The test material did not have specific developmental effects in this study.

Reliability : (2) valid with restrictions

Published reports are assigned a reliability of 2. Despite differences from the current guideline and the lack of details that would be reported in a modern investigation, the study appers to have been well conducted and the data appear to be robust.

Flag : Critical study for SIDS endpoint
10.12.2003 (21)

Species	:	mouse
Sex	:	female
Strain	:	other: CLFP (ICI Strain 2) outbred
Route of admin.	:	gavage
Exposure period	:	day 6-15 of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	125, 250, 500 or 1000
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 500 mg/kg bw
NOAEL teratogen.	:	= 500 mg/kg bw
Result	:	Not specific developmental toxin
Method	:	other: EPA Guideline 83-3, OECD 414 Draft
Year	:	1984
GLP	:	yes
Test substance	:	

Method : Groups of 40 female SPF CLFP (ICI Strain 2) outbred mice (weight range 26 to 42.9 grams) that has been time-mated to males of the same strain were treated by gavage with Technical Biphenyl in corn oil dosed from day 6 to 15 of pregnancy. Dose levels, selected based on a preliminary study, were 0, 125, 250, 500 or 1000 mg/kg body weight. Animals were weighed on day 1, 3, 6, 8, 10, 14 and 17 of pregnancy. Food consumption was determined as a function of the weighing intervals. Animals were sacrificed on gd day 17.5 cervical dislocation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs, the ovaries and uteri were examined immediately to determine: number and distribution of live young, number and distribution of embryofoetal deaths, individual fetal weights, fetal abnormalities.

Live young were examined externally and weighed. Half the fetuses in each litter were preserved is Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique. The remainder were fixed in 74-OP industrial methylated Sprit for subsequent macroscopic examination, evisceration, clearing and alizarin staining for skeletal examination. All fetuses were sexed by gonadal inspection following preservation.

Statistical Analysis: Statistical analysis were routinely performed on litter data using a two-tailed test for significance at the 0.05 level. Non-parametric tests are primarily used due to non-normal distributions of most parameters. Mean values of litter size, post-implantation loss, litter weight, mean pup weight and the incidence of anomalous offspring were analyzed by the Jonckheere and Kruskal-Wallis tests. Fisher's exact test was employed where a high incidence (75%) of tied values occurred. Incidence values for maternal mortality and total resorption were also analyzed using the Chi-Square test.

Result :

Maternal Effects: There was a high incidence of non-pregnancy in all groups (the reason for the large group size) and it was not related to the test material. In the animals receiving the highest dose, 1000 mg/kg, it was found that total resorption occurred in seven litters. This resulted in an overall reduction in maternal weight gain but no reduction if only animals bearing live pups are considered. Food consumption was similar in all groups and controls. Maternal mortality was increased at the high-dose level and reported as 0, 0, 1, 2, and 8 in control to high dose, respectively. No clear cause of death was discovered at necropsy of the decedents. No clear treatment-related effects were seen at terminal sacrifice.

	GROUP (mg/kg)				
	0	125	250	500	1000
Mated	40	40	40	40	40
Sacrificed	0	0	1	0	4
Died	0	0	0	2	4
Tot mortality	0	0	1	2	8**
Non-Pregnant	17	16	19	18	15
Total resorption	1	0	3	4	7**
With live young	22	24	17	16	10

** < 0.01 Chi Square Test

Litter Effects: Total resorptions were significantly increased in the high-dose group and the incidence was 1, 0, 3, 4 and 7, control to high dose. Mean litter size was also reduced at the high dose but this was entirely due to the 7 dams resorbing the entire litter. Mean litter and fetal weighs were similar in all groups. Sex ratio was not affected by treatment.

Malformations: The incidence of malformed fetuses was 3, 6, 8, 3 and 4 from control to high-dose. Neither the type nor distribution suggested an association with treatment.

Variations: There were slight intergroup differences in mean incidence of fetuses with extra ribs or variant sternebrae but these were not suggestive of a treatment-related effect.

Test substance :

Biphenyl, Technical. CASNO 92-52-4

Conclusion :

Biphenyl was clearly fetotoxic and maternally toxic at 1000 mg/kg causing mortality of both dams and early-pregnancy loss including complete resorptions. The 500-mg/kg dose level was statistically a NOAEL for both dams and fetuses. In spite of the fetotoxicity and maternal toxicity the incidence of malformations was not increased.

Reliability : (1) valid without restriction

Modern guideline study under GLPs with clear maternal toxicity achieved.

Flag : Critical study for SIDS endpoint

10.12.2003 (1)

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